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THE USE OF ANTI-*OSTERTAGIA OSTERTAGI* ANTIBODIES IN MILK TO STUDY THE EPIDEMIOLOGY AND IMPACT ON PRODUCTION OF GASTROINTESTINAL-NEMATODE INFECTIONS IN DAIRY COWS

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List of abbreviations

AUC	Area under the curve
BL	Bovine leukaemia
BVD	Bovine viral diarrhoea
CCC	Concordance correlation coefficient
CI	Confidence interval
C_{max}	Maximal concentration
DIM	Days in milk
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EPG	Eggs per gram
EU	European Union
FEC	Faecal egg count
FSG	First-season grazing
GI	Gastrointestinal
GIS	Geographical information system
IBR	Infectious bovine rhinotracheitis
Ig	Immunoglobulin
Inj.	Injectable
L_1	First-stage larva
L_2	Second-stage larva
L_3	Third-stage larva
L_4	Fourth-stage larva
LL	Lower limit
MAP	*Mycobacterium avium* subspecies *paratuberculosis*
MCC	Milk Control Centre
ML	Macrocyclic lactone
NC	Negative control
OD	Optical density
ODR	Optical-density ratio
PBST	Phosphate-buffered saline with 0.05 % Tween 20
PC	Positive control
RS	Remote sensing
UL	Upper limit
V.R.V.	Flemish Cattle Breeding Association

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General introduction

1. Introduction

Infection of livestock with gastrointestinal (GI) parasitic nematodes is a major constraint on production worldwide. All grazing cattle are exposed to infection with these parasites resulting in economic losses (mainly due to reduced productivity and costs of anthelmintic treatments). Traditionally, infections with GI nematodes were considered to be mainly important in first-season grazing (FSG) calves. The effects of clinical disease in this age group have been clearly demonstrated and are well known to both farmers and practitioners. In contrast, infections with GI nematodes in older cattle were for a long time considered to be of no major importance because of the absence of clinical symptoms and the lower fecal egg counts usually found in these animals. However, recently 2 reviews have demonstrated that subclinical GI-nematode infections can impair milk yield (Gross *et al.*, 1999; Sanchez *et al.*, 2004). This, together with the availability of highly efficacious anthelmintics with a zero withdrawal time for milk has generated new interest for GI-nematode control in adult dairy cows. In the following paragraphs, a short review will be given on the prevalence, life cycle, epidemiology and pathology in the northern hemisphere of *Ostertagia ostertagi*, the most important GI nematode of cattle in temperate-climate regions.

2. Gastrointestinal-nematode species and their relative importance in dairy cattle

In temperate-climate regions, GI nematodes are widespread in dairy cattle. Several abattoir studies in the United States and Western Europe have shown that the prevalence of infection in adult cows is between 80-100 %. This is not unexpected, because all pastures are likely to be contaminated, and so all grazing animals are likely to be exposed to infection.

The most important species in temperate climate areas are *O. ostertagi* and *Cooperia oncophora*. The relative importance and infection levels of the genera differ with host age because of acquired immunity.

Table 1. The important GI nematodes of cattle in temperate-climate regions per age category

Localisation	Calves	Adult Cows
Abomasum	*Ostertagia ostertagi*	*Ostertagia ostertagi*
	Trichostrongylus axei	*Trichostrongylus axei*
Small intestine	*Cooperia oncophora*	
	Nematodirus helvetianus	
	Trichostrongylus colubriformis	
Large intestine	*Oesophagostomum radiatum*	

A strong host resistance develops within 1 year to most species. *Ostertagia* engenders immunity more slowly and is therefore the most important species in older cattle (Table 1). Burdens of *Ostertagia* in adult dairy cattle vary from zero to over 200,000 worms. In Europe, the frequency distribution within herds is as follows: about 80-85 % of the animals harbour a low worm burden (0–10,000 worms) and about 15-20 % harbour a high burden (>10,000 worms). The majority of these worms are present as hypobiotic fourth-stage larvae (L_4), which induce limited pathology (Vercruysse *et al.*, 2002).

3. Life cycle and epidemiology of *Ostertagia ostertagi*

Ostertagia has a direct life cycle which consists of a free-living phase on pasture and a parasitic phase in the host (Fig. 1). *O. ostertagi* infects the abomasum of cattle. The eggs of the parasites are passed in the faeces and develop in the faecal pat to the first-stage larva (L_1). After a first moult, the second-stage larva (L_2) emerges. Next, the infective third-stage larva (L_3), still enclosed in the loosely-fitting L_2 cuticle (sheath) will develop. Under optimal conditions of temperature and humidity, the eggs can develop into infective L_3 within 2 weeks. Ingestion of grass contaminated with the infective L_3 causes the actual infection. After ingestion, the L_3 exsheath in the rumen and may penetrate the gastric glands of the abomasum within 6 h after ingestion. They develop to L_4, and then emerge into the abomasal lumen where they establish themselves as adults. The normal prepatent period is approximately 21 days, but under some conditions, many of the ingested L_3 become

arrested in their development at the early fourth stage for a period of up to 6 months.

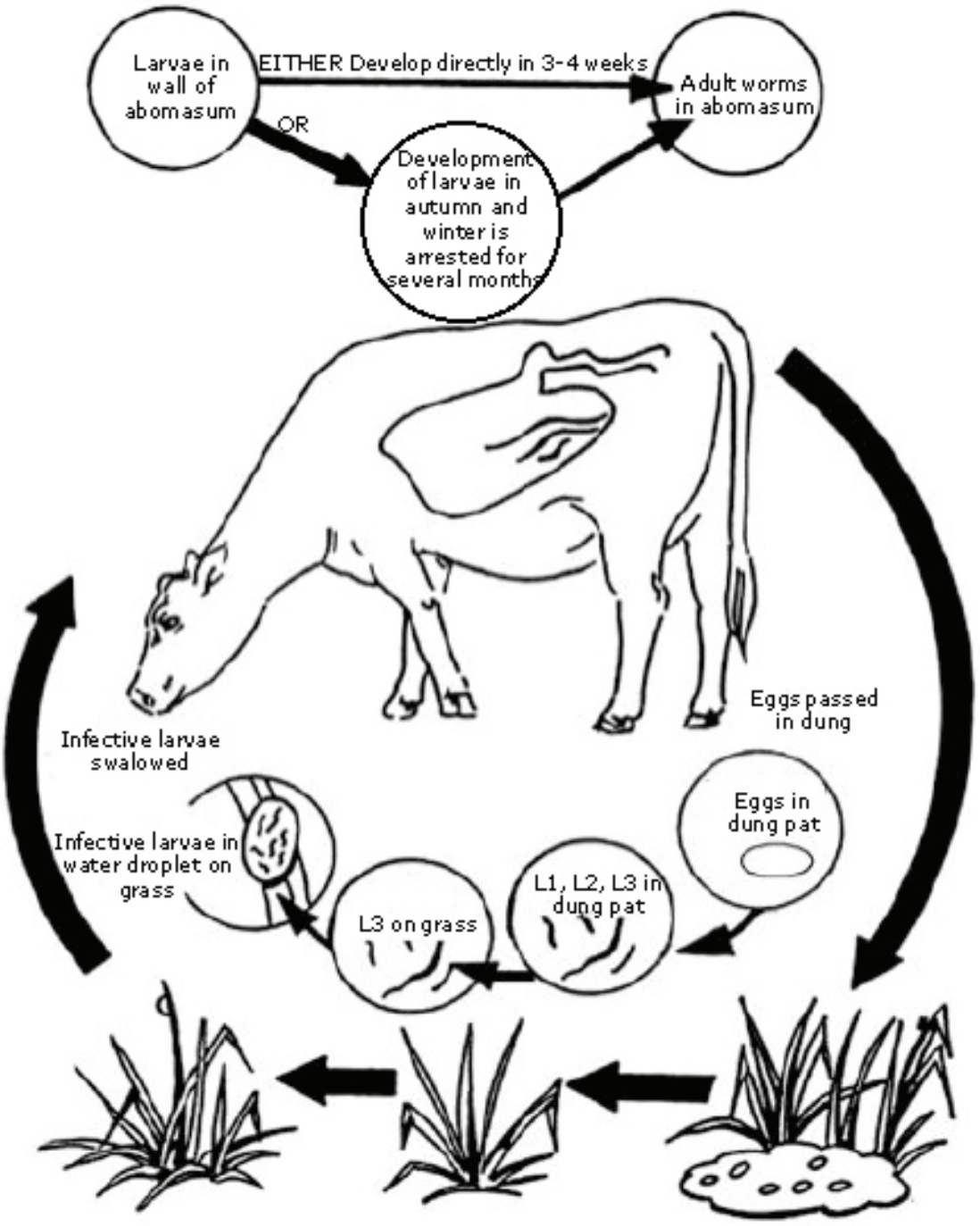

Fig. 1. Life cycle of *Ostertagia ostertagi,* adapted from Petalia ™ & © 2000 Petsite.com Ltd; http://www.petalia.com.au

Nematode infections in spring are derived largely from over-wintered larvae on pasture but in some cases may consist of a few over-wintered adults or worms maturing from inhibited larvae. Infective larvae are ingested by animals that are turned out in the beginning of the new

grazing season (May/June) (Fig. 2). After 3 weeks, eggs are shed and they develop to L_3. At this time of year, the hatching of the eggs is rather slow but it becomes more rapid towards mid-summer as the temperature rises. The majority of the eggs deposited during April, May and June will reach the infective stage from mid July onwards. This is termed the "mid-summer rise". The exact period in which this occurs is variable and depends on the weather. When the summer is very dry, larvae will accumulate in faecal pats and will not much migrate out of it. These larvae however will emerge when wet weather returns in the autumn. This will lead to a very high pasture contamination. When temperatures fall and autumn progresses, an increasing proportion of ingested L_3, will only develop to the L_4 stage and then go into arrested development. In late autumn, calves can therefore harbour thousands of these "early L_4" but few developing forms or adults.

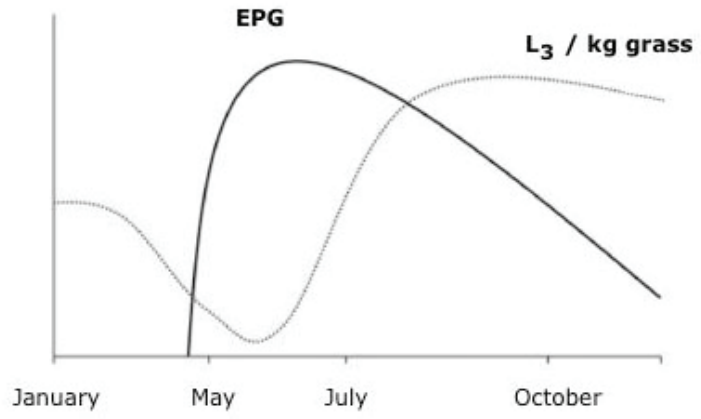

Fig. 2. Diagram showing the egg output versus the pasture contamination of *Ostertagia*. EPG: eggs per gram faeces

4. Pathogenesis and pathology of ostertagiosis

The presence of nematodes in the abomasum in sufficient numbers induces structural, biochemical, hormonal, nutritional and immunological changes. When infective larvae penetrate the gastric glands these become dilated and enlarged (Fig. 3). During the growing process of the larvae inside the glands, the parasitized mucosal glands become distended and the common cells lining the abomasum such as zymogenic cells, mucous

cells and parietal cells are replaced by undifferentiated epithelium (Murray *et al.*, 1970). After these replacements an elevation of the pH of the abomasal contents is seen. For this reason, pepsinogen is not converted into pepsin (McKellar, 1993). Because of the elevated pH, bacteriostatic activity and peptic digestion are decreased in the abomasum. When adult parasites emerge from the glands, the junctions between epithelial and endothelial cells are ruptured with an increased plasma pepsinogen level and plasma protein loss as a result (McKellar, 1993). All these changes in cell structure and coherence can cause clinical signs (ostertagiosis).

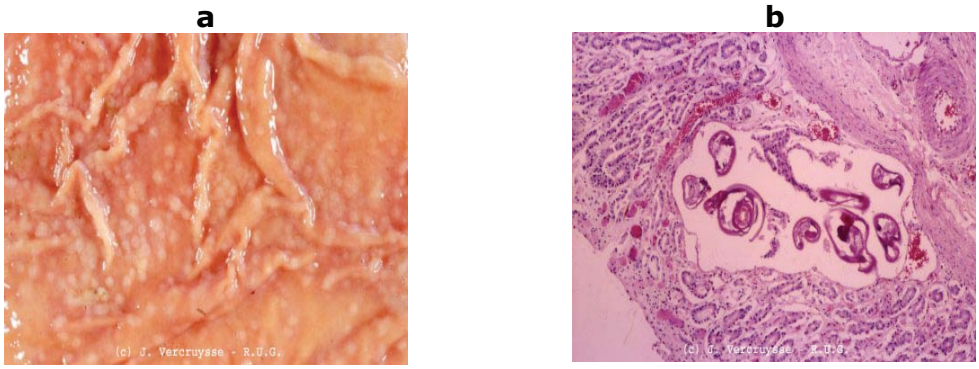

Fig. 3. a. Dilated and enlarged gastric glands after infection of L_3. b. Transversal section of an infected gastric gland.

Traditionally, 2 forms of clinical ostertagiosis were described: (a) Type I ostertagiosis, caused by immature adults leaving the gastric glands, that occurred typically between July and October; and (b) Type II ostertagiosis, caused by arrested L_4 that resume their development to immature adults and leave the gastric glands weeks or months after first being ingested as L_3, that occurred during the late fall and winter months. These clinical forms of ostertagiosis can occur in FSG calves when no appropriate preventive measures against GI-nematode infections are taken and are manifested by (watery) diarrhoea, weight loss, a dull hair coat, anorexia, a general loss of condition and eventually death. However, in the past 25 years, anthelmintic-drug development and the strategic use of anthelmintics have brought about that ostertagiosis in cattle is now mostly observed to be subclinical and both types (I or II) of the disease have become rare. The main manifestation of subclinical ostertagiosis in calves is a reduced growth performance.

In older cattle, infections with GI nematodes were for a long time considered to be of limited importance because of the absence of clinical symptoms and the lower fecal egg counts usually found in these animals. However, it has been demonstrated that subclinical GI-nematode infections can have a negative impact on milk yield (Sanchez *et al.*, 2004).

Although the herd's performance can be considerably affected by subclinical parasitism, little is known about the possible mechanisms of this impairment. The most relevant effect of GI parasitism on dairy cattle is the loss of appetite caused by an infection. It was observed that dewormed cows and heifers grazed 50 min longer per day than untreated controls. The reduced appetite may be a result of the increased gastrin levels associated with the increased abomasal pH, which is in turn a result of damage to the parietal cells. Other reported effects of GI parasitism are alterations in the GI-tract motility, in GI secretions and in digestion and absorption (Fox, 1997).

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CHAPTER 1

The diagnosis, impact on production and control of gastrointestinal nematodes in dairy cattle

1. Introduction

The European Union (EU) is the world's largest producer of cow milk, followed by the United States and South-America. In 2005, the EU produced 121 million tons of milk or 19 % of the world milk production. Traditionally, dairying is one of the most profitable sectors of EU agriculture. Although the family farm income per unit of unpaid labour in specialist dairy farms decreased from the mid-1990s from € 25,500 to € 18,800 in 2003, it remains higher than the € 16,400 average for all types of farms (OECD-FAO, 2006). The average produce price for milk is about € 30 per 100 kg. This figure consists of both the market price and the EU subsidies for milk production. However, the current EU policy is to reduce its price support and allow a free-trade market. Therefore, it is expected that the milk price will decrease and show larger fluctuations than in the past. In response, the EU dairy production follows a trend toward increased intensification on a smaller number of larger, more specialised production units (van Arendonk and Liinamo, 2003). For instance, in Flanders the number of dairy farms has decreased between 1995 and 2004 by 30 % to 8,757 production units, but the milk production is maintained by significant increases in herd size (+ 10 %) and milk production per cow (+ 30 %) (VILT, 2005). Dairy farmers have a clearly defined maximum output level (quota determined) and their goal is to produce this at the lowest possible cost. For the producers that account for the majority of the EU production this has resulted in maximising the production output per cow via high input: high output systems. High input: high output farming systems account for 83 % of the total EU dairy cow numbers and 85 % of the total EU milk production (van Arendonk and Liinamo, 2003).

Veterinary medicine is co-evolving with the evolutions in the dairy industry. In the last 25 years, there has been a paradigm shift from treatment of clinical illness to disease prevention (Leblanc *et al.*, 2006). A fundamental advancement has been the recognition of the multifactorial nature of almost all diseases of importance in dairy cattle. In turn, health management has become characterized by an integrated, holistic, data-based and economically-framed approach to prevent disease and enhance

performance. There has been a shift in focus from individual animals to groups and herds.

Another major evolution has been redefining disease more broadly, to include subclinical conditions. This expansion has resulted both from improved diagnostic technology as well as from the evolution of health management in which any factor that limits animal or herd performance might be considered as a component of disease. Due to these evolutions, veterinarians are evolving from task-oriented providers of therapy to advice-oriented consultants. A new task of veterinarians is to collect quantitative data and analyze records in an effort to identify production-limiting problems at an early stage and assist the producer in achieving performance targets. The current health advisors must be able to install monitoring programmes and a decision/action plan to reach these targets (Leblanc *et al.*, 2006).

Whereas in the domains of nutrition and housing veterinarians must compete with other health advisors, they remain the specialists in the domain of infectious diseases. Major enzootic infectious diseases affecting dairy productivity are considered to be those causing mastitis and lameness, fasciolosis, bovine viral diarrhoea (BVD), dictyocaulosis, paratuberculosis and infectious bovine rhinotracheitis (IBR) (Bennett *et al.*, 1999). Currently, organised herd-health monitoring programmes are (being) established for mastitis, BVD, paratuberculosis and IBR. Future developments will probably imply the incorporation of other infectious diseases in the health-monitoring programmes. A promising candidate for incorporation in these programmes is infection with GI nematodes. This is especially so because highly efficacious anthelmintics are available to control the infection. Monitoring of GI nematodes requires knowledge on the available diagnostic tests and their characteristics. Moreover, it would only be useful if the applied control measures result in a substantial economic profit. Therefore, this chapter deals with the diagnosis, the impact on production and the possible control options of GI-nematode infections in adult dairy cows.

2. Diagnosis

2.1. Introduction

Veterinary care of domestic ruminants has evolved from treating sick individual animals to a herd-based approach that focuses on disease prevention (Leblanc *et al.*, 2006). Many producers are now willing to pay for scheduled visits to the farm by a veterinarian in the absence of an emergency or a clinically ill animal. This shift from treatment to prevention and advice requires good records of all animals in a herd and software to analyze these. Furthermore, suitable parameters have to be available for decision-making. Eysker and Ploeger (2000) stated that the main requirements of parameters to monitor GI-nematode infections should be that: (1) they enable an estimate of nematode exposure; (2) values reflect production losses from these infections; (3) they can be used to predict the risks of future production losses and allow recommendation of appropriate preventive measures; (4) they are easy to assess and (5) they are inexpensive.

Another major evolution in veterinary medicine was the recognition of subclinical disease as a limiting factor on productivity (Leblanc *et al.*, 2006). Whereas the diagnosis of clinical ostertagiosis can be made based upon anamnesis and clinical signs, other diagnostic parameters have been evaluated to detect subclinical infections. The available diagnostic markers have been shown to be especially useful in FSG calves. However, a major problem was that most of the used parameters were of little value in adult cows.

2.2. Faecal examination

Faecal egg counts (FECs) are the most widely used parameter in studies on GI-nematode infections of young ruminants. The reason is that it is an easily applicable and low-technology parameter. FECs are especially useful for use in FSG calves, to assess in the middle of the grazing season whether "low" or "high" initial infections occurred and subsequently to decide on further worm control measures (Ploeger *et al.*, 1994; Shaw *et al.*, 1998a). A limitation of FECs is that they reflect the egg output of adult female worms only. When samples are taken in the second half of the

grazing season, the correlation between FEC and infection level becomes faint because the calves acquire immunity to the GI nematodes and this causes a reduction of egg production by the worms (Claerebout and Vercruysse, 2000). Due to the acquired immunity, the egg output in adult cows is generally low and a large proportion of *Ostertagia ostertagi* in adult cows is present as inhibited L_4. Consequently, FECs are considered as a poor indicator to detect the presence or quantify the infection level of GI nematodes in adult cattle (Michel, 1968). In 2 abattoir surveys, (Agneessens *et al.*, 2000; Borgsteede *et al.*, 2000) 94-96 % of the cows were infected with GI nematodes on abomasal worm counts and nematode eggs were detected in the feces by coproculture in 64-89 % of the animals. However, the geometric mean number of eggs per gram (EPG) was only 2.4 and the majority of the cows (81-86 %) had an EPG below the detection limit of a routine FEC technique. Moreover, no relationship between anthelmintic-treatment response on milk yield and infection level could be demonstrated using FECs as a parameter of infection (Michel *et al.*, 1982; O'Farrell *et al.*, 1986). Another problem with FECs is that the repeatability is low, especially when the number of eggs per gram is low and only a small volume of faeces is analysed (Gasbarre *et al.*, 1996; Cringoli *et al.*, 2004).

2.3. Serum-pepsinogen determination

The development and emergence of larval stages of *Ostertagia* causes mucosal damage with hypo- and metaplasia of the parietal cells resulting in a decrease of acid production and a subsequent reduction of the pepsinogen transformation into pepsin. The accumulated pepsinogen may escape into the blood between the broken cell junctional complexes (Berghen *et al.*, 1993). An increase in serum pepsinogen concentration is mostly caused by an *Ostertagia* infection. However, other parasitic or non-parasitic diseases can also be responsible for a moderate rise in pepsinogen concentration (e.g. *T. axei*, abomasal ulcerations) (Ross *et al.*, 1967; Vörös *et al.*, 1984).

It has been demonstrated that serum pepsinogen concentrations correlate well with infection levels of abomasal nematodes (Berghen *et al.*, 1993) and they are therefore considered as a very useful tool to monitor the

Ostertagia-infection status in FSG calves. Dorny *et al.* (1999) observed a reasonable relationship (R^2= 0.71) between individual pepsinogen values determined at housing and adult *Ostertagia* burdens obtained at slaughter. However, in adult cows the value of the serum pepsinogen determination is considered poor. Elevated pepsinogen values can be observed in clinically healthy cows (Berghen *et al.*, 1988), probably as a result of hypersensitivity acquired during infections in previous years. In addition, no correlation was found between the effect of anthelmintic treatment on milk yield and the *Ostertagia* infection level as estimated by serum pepsinogen concentration (O'Farrell *et al.*, 1986; Ploeger *et al.*, 1989).

2.4. Measurement of specific antibodies

Enzyme-linked immunosorbent assays (ELISA) to measure *O. ostertagi*-specific antibody levels have been used in epidemiological studies since the 1980's (Keus *et al.*, 1981). The assay quantifies the levels of immunoglobulines (Ig) G that react with crude adult-worm extracts of *O. ostertagi*. *Cooperia oncophora* ELISAs have also been evaluated but have shown only promising results in evaluating the exposure level in FSG calves (Ploeger et al., 1994).

The first epidemiological studies evaluating the *Ostertagia*-specific antibody levels in serum indicated that the antibody levels are not an accurate indicator of an active infection or of the size of the worm population. However, they reflect the uptake of L_3 from pasture or the resuming development of arrested L_4 (Kloosterman, 1983; Ploeger *et al.*, 1995; Claerebout *et al.*, 1997). *Ostertagia*-specific IgG levels in serum reflect the interaction between the acquired immunity and the antigenic stimulation to which the animal is currently exposed, rather than the immune status or the infestation level alone. This is called the cumulative exposure (Claerebout, 1998). However, in the continuation of this thesis, this will also be referred to as "infection level".

Ploeger *et al.* (1989, 1990a, b, c, d, e, f) observed a significant between-herd variation in the mean serum antibody titres against *Ostertagia* of calves and adult cows, suggesting that it is possible to estimate the infection level in a dairy herd by examining serum antibody titres.

Moreover, herd-mean *Ostertagia*-specific antibody titres could be related to several production parameters. When measured at the end of the first grazing season, they were negatively correlated with the weight gain during the following winter-housing period (Ploeger *et al.*, 1990b, d). At the end of the second grazing season, they were negatively correlated with the weight gain over the finished grazing period (Ploeger *et al.*, 1990e). In adult cows, it was observed that the mean herd milk-production response to anthelmintic treatment was correlated positively with the mean herd *Ostertagia* antibody titre (Ploeger *et al.*, 1989).

A disadvantage of a crude-antigen ELISA is that cross-reactions with other helminths may occur. Cross-reactions of the *O. ostertagi* ELISA with antibodies against *Cooperia* spp. have been demonstrated (Keus *et al.*, 1981) but are not considered as a disadvantage because the aim is to estimate the overall GI-nematode infection, rather than just *O. ostertagi* infections. Cross-reactions with *Dictyocaulus viviparus* and *Fasciola hepatica* have also been suggested and may pose some difficulties (Ploeger *et al.*, 1994). However, attempts to develop a more specific ELISA have so far only succeeded for *Cooperia* spp. (Poot *et al.*, 1997) and this assay is only useful for evaluating the exposure level to GI nematodes of calves in their first grazing season (Eysker and Ploeger, 2000; Githiori *et al.*, 2000). A highly-specific *O. ostertagi* antigen was identified by de Graaf *et al.* (1994). However, no relationship existed between the exposure to L_3 and the ELISA results, hindering the application of this ELISA as a diagnostic tool.

Overall, these first studies indicated that antibody levels against crude adult *O. ostertagi* antigen had potential as a diagnostic parameter for GI-nematode infections in cattle. Moreover, it was the only diagnostic parameter showing promise for use in adult dairy cows.

In adult dairy cows, research focuses on the detection of anti-*O. ostertagi* antibody levels in (individual or bulk-tank) milk because this medium is less costly and therefore more suitable for routine-screening purposes. The repeatability between plates of an *O. ostertagi* ELISA was evaluated, showing that expressing the test results as an optical-density ratio (ODR) gave the most repeatable results (Sanchez *et al.*, 2002b), where ODR= [optical density (OD) of the sample – OD negative control] / [OD positive

control – OD negative control]. In addition the coated microtitre plates proved to be stable over time (Sithole *et al.*, 2005b), indicating the feasibility to develop this test as a standardised, commercial assay.

It was demonstrated that the serum antibody level is by far the most influential factor in determination of the milk antibody level (Kloosterman *et al.*, 1993). However, other factors such as milk yield, age, stage of lactation and somatic-cell count also influence the milk antibody levels. The ODRs of individual cows follow the same variation across lactation as the total IgG levels (Sanchez *et al.*, 2004b). They correlate positively with days in milk, age and somatic-cell count. A small negative relationship exists between the ODR and daily milk yield (Sanchez *et al.*, 2004b).

Despite these variations across lactation, significant relationships were found between anti-*Ostertagia* bulk-tank milk antibody levels and certain management practices known to be associated with GI-nematode infection levels (e.g. level of exposure to pasture, anthelmintic treatment), suggesting that bulk-tank milk antibody levels are a reasonable measure of the parasite-infection level in a dairy herd (Guitián *et al.*, 2000; Sanchez and Dohoo, 2002).

One of the remaining problems is to assess if the *O. ostertagi* ELISA can be used to identify the animals/herds where GI-nematode infections are impairing productivity. Preliminary investigations on a sub-set of 123 cows from a study on 27 farms in Canada found an overall response to anthelmintic treatment of 1.3 kg milk/day over the first 6 months of lactation. However, whilst low-ODR (< 0.5) cows showed no response (0.1 kg/day), high-ODR (> 0.5) cows responded by 2.9 kg/day. The association between precalving ODR and response to anthelmintic treatment was marginally significant (Sanchez *et al.*, 2002a). In a subsequent study in 30 Canadian dairy herds with limited outdour exposure in which cows were treated at calving, there was no overall treatment effect on milk yield. However, there was a significant interaction between the individual pre-calving ODR and treatment response with some evidence of a positive response in cows with an ODR> 0.4 (Sanchez *et al.*, 2005). On the herd level, the usefulness of bulk-tank milk remains unclear. In a study on 120 dairy herds in Canada, a negative relationship was established between the anti-*Ostertagia* antibody level in

bulk-tank milk and the annual milk yield (Sanchez and Dohoo, 2002). In addition, higher milk-yield responses to anthelmintic treatment were observed in herds with a high bulk-tank milk antibody level than in the low bulk-tank milk antibody level herds. However, significant differences could not be demonstrated (Kloosterman *et al.*, 1996; Sithole *et al.*, 2005a) and this area needs further investigation.

2.5. Other techniques

In the past, serum-gastrin determination has been proposed as a potential diagnostic for ostertagiosis. An increase in gastrin levels in calves occurs around patency of *Ostertagia* infections (Fox *et al.*, 1989) and seems to be associated with the presence of adult worms (McKellar *et al.*, 1986). The main advantage of gastrin values over pepsinogen values seems to be that they are not influenced by hypersensitivity responses to incoming infective larvae (Ploeger *et al.*, 1994). However, gastrin is only elevated at very high infection levels. It was shown experimentally that only a high infection dose (> 100,000 L_3) administered to parasite-naïve calves provoked a significant gastrin release (Berghen *et al.*, 1993). In adult cows experimentally infected with 100,000 *Ostertagia* L_3, no gastrin response was evoked, probably because of the development of only a small adult-worm population in the immunized host (Pitt *et al.*, 1988). It was concluded that gastrin determination is suitable for confirming an outbreak of clinical ostertagiosis, but not for the detection of lower infection levels that induce subclinical losses (Eysker and Ploeger, 2000). The technique is also expensive and is therefore not considered as a useful tool for herd-health monitoring.

Other diagnostic techniques are still in an experimental phase. A copro-antigen detection ELISA was developed by Agneessens *et al.* (2001). However, the specificity and the correlation between the results of a copro-antigen detection test and the number of *Ostertagia* worms were too low to allow a practical applicability of the test. Detection of worm DNA is not expected to be suitable for monitoring the GI-nematode infections because the test should be quantitative and the only obvious sources for DNA are the eggs in the faeces. Considering that FECs are not a good parameter for diagnosis, this automatically disqualifies a DNA-

based test for adult cows (Eysker and Ploeger, 2000). The detection of genetic markers that indicate the susceptibility of the animal to GI-nematode infections seems a more promising approach. It is often observed that only a small proportion (± 25 %) of the animals harbour a high worm burden, while the other animals harbour a low worm burden. It has been demonstrated that this infection pattern is strongly influenced by the host genetics (Gasbarre *et al.*, 1990). It is anticipated that if the highly-susceptible animals can be identified by a genetic marker, effective control strategies can be developed based on targeting drug administration to the small percentage of highly susceptible animals (Gasbarre *et al.*, 2001). However, some promising genetic markers have until now only been identified in sheep (Beh *et al.*, 2002) but not in cattle (Sonstegard and Gasbarre, 2001).

3. Impact on production

3.1. Introduction

The economic losses associated with GI parasitism in cattle are universally accepted. Many trials have been conducted to determine the effects of GI nematodes on production traits. These are mostly estimated by measuring the association between infection and production parameters or by assessing the effect of anthelmintic treatment on production. Economic studies estimating the monetary costs of the production losses or the costs and benefits of possible control measures are scarce. Possible reasons may be (1) the incomplete information on the extent to which production is adversely affected by infections; (2) the variation in importance of productivity indices from place to place and (3) the multicausal nature of production losses which make it difficult to tease out those components of the loss that are attributable to GI parasitism (Smith, 1997; Vercruysse and Claerebout, 2001). Therefore, the following paragraphs are a review on the reported effects of GI-nematode infections in cattle on productivity, without converting these into a monetary value. A special emphasis will be given on studies that evaluate the effect of GI nematodes on milk yield.

3.2. Effect on weight gain and carcass quality

The most obvious and most frequently measured benefit of parasite control is gain in body weight. This increase in body weight has been demonstrated in many different classes of cattle and under many different types of management and geographic locations. Most documented are the effects of anthelmintic treatments on weight gain in FSG cattle. Shaw *et al.* (1998b) performed a meta-analysis of 85 studies on the weight gain during the grazing period involving over 2,000 FSG calves over a 26-year period in 13 countries in Western Europe. The GI-nematode infections were classified as "subclinical" or "clinical" depending on the observation of clinical signs of parasitic gastroenteritis in the control groups. Average weight gains in the clinical control groups were 375 g/day. The average weight gain in chemoprophylactic treated calves with subclinical infections was 690 g/day vs. 540 g/day in untreated control calves with subclinical infections. GI-nematode infections can also cause a decreased growth rate during the housing period. The average weight gain during the housing period correlated negatively with infection parameters that estimate the level of exposure during the past grazing season (Ploeger *et al.*, 1990a, d; 1995). Trials on 32 and 48 farms in the Netherlands, evaluating the effect of anthelmintic treatment on weight gain during the housing period found an average effect of 59 g/day and 36 g/day, respectively (Ploeger *et al.*, 1990a, d).

The effect of anthelmintic treatment on growth has also been studied in second-season grazing animals of both dairy and beef breeds. Investigators consistently report increased weight gains and a subsequent reduction in time to reach breeding weight in replacement heifers (Hawkins, 1993). Interestingly, Ploeger *et al.* (1996) observed that the infection-induced differences in weight gain during the first grazing season appeared to be permanent, at least up to the end of the second grazing season. Moreover, first-lactation yield was positively correlated with body weight at calving. This suggests that nematode infections occurring in the first 2 years of life negatively influence milk production by reducing weight gains.

Also carcass quality can be affected by GI-nematode infections. Entrocasso *et al.* (1986) reported increased carcass weight, killing out

percentage and related carcass measurements in anthelmintic treated steers.

3.3. Effect on milk production

3.3.1. Studies

The effect of GI nematodes on milk production is a topic that has been the focus of much research effort over the last 3 decades. Overall, the studies can be divided into (1) studies evaluating the effect of experimental infections; (2) clinical trials evaluating the effect of anthelmintic treatment; (3) surveys investigating the relationship between *Ostertagia*-specific antibody levels and milk yield.

The results of studies of experimental infections should only be extrapolated to field situations with caution. It is very difficult to reliably simulate the infection pattern of animals under natural grazing conditions. Moreover, the cows included in such studies are mostly not representative for the "average" cow because they have often an unknown and varied history and low production level (Gross *et al.*, 1999). Most studies belong to the second category and evaluate the effect of anthelmintic treatment on milk yield. They have the advantage that they are applied in real farm situations and that they provide an estimate of the avoidable production loss (Perry and Randolph, 1999). However, no standard approach for evaluating the effect of anthelmintic treatment on milk yield has been adopted by researchers. The quality (randomisation, blinding, control for confounders) and design of the trials vary considerably. Differences in the method of controlling confounders, the period of time milk production was followed up and the use of different milk production measures (e.g. daily weight, solids-corrected, 305-day projected) often hamper a comparison of the results from different trials. Another problem is that the used anthelmintics have often a broad-spectrum activity and it is therefore not known which part of the observed effects can be attributed to the removal of the GI worm burden. The studies from the third category (surveys) have the advantage that they can be performed on a larger scale and relate diagnostic test outcomes with productivity. However, they cannot

be used to demonstrate a causal relationship between the presence of GI nematodes and a reduced milk yield.

3.3.2. The effect of experimental infections

Bliss and Todd (1977) found that cows given a single infection with 200, 000 mixed species trichostrongylid larvae produced 1.2 kg/cow per day less than uninfected controls during 30 days following the inoculation. Barger and Gibbs (1981) simulated the natural exposure of grazing cows by a trickle infection with 5,000 mixed species larvae during 9 weeks and followed the milk production during the same period. The uninfected cows produced 2.2 kg/cow per day more than the experimentally infected cows. However, because of the small number of animals in the trials, significance of the results was either not reported or not reached. Moreover, 3 other studies observed no effect of dosing infective larvae on milk yield (Kloosterman and Albers, 1982; Kloosterman *et al.*, 1985; Pitt *et al.*, 1988). Therefore, the results from trials with experimental infections were inconclusive and the effect of GI nematodes on milk yield was further investigated by assessing the effect of anthelmintic treatment on milk yield.

3.3.3. The effect of anthelmintic treatment

A whole spectrum of different anthelmintics has been evaluated going from coumaphos in the 1970's, over benzimidazols and levamisol in the 1980's to ivermectin in the 1990's. Gross *et al.* (1999) performed a narrative review of these trials (n= 87). The studies were divided into 4 general categories: (a) induced infections into previously uninfected cattle; (b) naturally infected cattle treated during mid-lactation; (c) naturally infected cattle treated in the dry period or in early lactation and (d) naturally infected cattle treated repeatedly from early lactation or given strategic treatments throughout the year. The results of these trials are summarized in Table 1.1. Overall, there was an increase in milk production after anthelmintic treatment in 80 % of the trials, with a median increase of 0.6 kg/cow per day.

Table 1.1. Proportion of trials with a positive production response after anthelmintic treatment, and median and range of the effect on milk yield from studies performed before 1997 (after Gross *et al.*, 1999)

Study category	Proportion positive	Median (kg/day)	Range (kg/day)
Treated with anthelmintic during mid-lactation	15/19	0.8	-1.3 to 2.1
Treated with anthelmintic in dry period or early lactation	34/43	0.4	-2.3 to 2.1
Treated with anthelmintic repeatedly from early lactation or given strategic treatments throughout the year	15/16	0.8	-0.4 to 3.2

In 1997, an anthelmintic with a zero-withdrawal time for milk, called eprinomectin was introduced to the market. Since then, a number of studies have been carried out in different geographical locations and according to different study designs to evaluate the effect of eprinomectin treatment on milk yield. These studies are summarized in Table 1.2. Most studies report a positive effect of treatment on milk yield. There is 1 study in which no effect was observed (Sithole *et al.*, 2005a). In this study, the herds had no or only a limited outdoor exposure. In the other studies, the effects on milk yield range from 0.4 kg/cow per day in continuously pastured herds in New Zealand to 2.1 kg/cow per day in a *Hypoderma* sp.-endemic area in Switzerland. The effect on milk yield was persistent over the time of follow-up in each study and no effect of treatment on the milk-solids content was observed.

Table 1.2. Studies evaluating the effect of eprinomectin treatment on milk yield

Reference	Number of cows/herds	Time of treatment	Treatment response[a]	Follow-up
McPherson *et al.*, 2001	849/3	Calving	0.4*	full lactation
Nødvedt *et al.*, 2002	901/28	Calving	0.9**	6 months
Sithole *et al.*, 2005a	4,789/64	Calving	-0.1 (NS)	200 days
Reist *et al.*, 2002	742/79	October-December	2.1**	305 days
Forbes *et al.*, 2004	40/1	June	1.1	30 days
Gibb *et al.*, 2005	24/1	3 treatments at 7-week intervals starting 1 month after turnout	1.7*	18 weeks

a: Treatment response in kg/cow per day; * $P< 0.05$; ** $P< 0.01$; NS= non significant.

As a conclusion, both the old and more recent studies demonstrate that anthelmintic treatment can result in a positive milk-yield response. A meta-analysis of 75 trials published between 1972 and 2002 estimated this response after controlling for small-study effect and publication bias, at 0.35 kg/cow per day (Sanchez *et al.*, 2004a). However, there are large differences in treatment responses between different studies and within studies between different herds. The production responses to treatment are greater in trials using endectocides instead of older anthelmintics and when whole-herd treatments or treatment in mid-lactation or strategically throughout the year were performed instead of calving or dry-period treatment (Sanchez *et al.*, 2004a). Despite this, a large part of the between-study variability remains unexplained and it has been suggested that differences in the level of infection with GI nematode could account for a large part of this variation.

3.3.4. The relationship between *Ostertagia*-specific antibody levels and milk yield

Surveys were performed in Canada to investigate the relationship between *Ostertagia*-specific antibody levels in bulk-tank milk and milk yield. The relationship was estimated by linear-regression models including a number of factors that are known to be related with milk yield (pasture access, days in milk (DIM), lactation number, somatic-cell count). In a survey on 147 herds, the *Ostertagia*-specific antibody level in bulk-tank milk was determined and expressed as ODs. The herd OD in autumn was not associated with either annual milk production or seasonal decline in milk production (spring/autumn). However, an increase of the OD over the interquartile range was associated with a reduction in the summer milk production of 1.3 kg/cow per day (Guitián *et al.*, 2000). In a survey on 191 farms, an increase in the fall *Ostertagia* ODR over the interquartile range was associated with a drop in the annual average milk production of 1.2 kg/cow per day (Sanchez and Dohoo, 2002).

3.4. Effect on reproductive performance

Little work has been published on the effect of GI nematodes on reproductive performance in dairy cattle. In studies of anthelmintic-

treated beef cows, compared with untreated controls, there have been reports of increases in cow conception rate, calving rate, reductions in calf mortality and reductions in the calving to breeding interval (Hawkins, 1993; Gross *et al.*, 1999). In dairy cows, only few documented studies are available. These studies performed anthelmintic treatments in the dry period or at calving and followed the cows until conception. In a clinical trial in Australia on 5 dairy herds (430 cows), it was found that cows treated with ivermectin during the dry period had a 4.8 days shorter calving-to-conception interval than the untreated controls (Walsh *et al.*, 1995). In a Canadian study on 20 dairy herds (549 cows), Sanchez *et al.* (2002c) found a marginally significant reduction of the calving-to-conception interval in eprinomectin treated (117 days) vs. placebo treated (126 days) cows. On a subset of 109 cows, the antibodies in milk against *Ostertagia* were measured and expressed as an ODR. Among the placebo-treated animals, the probability of conception was significantly lower for high-ODR cows compared to low-ODR cows, suggesting that higher parasite infection levels had an adverse effect on reproductive performance. They also found a significant reduction in the number of breedings to conception in eprinomectin treated animals (1.7 vs. 1.9), but no effect on calving-to-first service interval. In contrast, a more recent study in Canada (35 herds, 2381 cows) could not find a beneficial effect of eprinomectin treatment at calving on reproduction (Sithole *et al.*, 2006). Moreover, no relationship existed between the *Ostertagia* ODR and reproduction parameters. The herds in this study had a limited outdoor exposure and the infection levels were assumed to be low. Nonetheless, until more studies on this subject are performed, the results on the effects of anthelmintic treatment on reproduction remain equivocal.

3.5. Effect on immunity

It has been demonstrated that during an infection GI nematodes can modulate the host's immune response, potentiating parasite survival (Claerebout and Vercruysse, 2000; Else, 2005). In addition, some studies suggest that *O. ostertagi* in cattle can provoke a non-specific immune suppression, reducing the ability of the animal to respond to heterologous antigens and increasing general susceptibility to disease (Wiggin and

Gibbs, 1989). A non-specific immunosuppression of lymphocyte blastogenesis in *Ostertagia*-infected calves has been demonstrated over an 8-week period (Snider *et al.*, 1986). Yang *et al.* (1993) observed a non-specific suppression of cellular and humoral immunity in *O. ostertagi* infected calves that were inoculated with *Brucella abortus* and IBR vaccines. Strategic anthelmintic treatments initiated better immune responses.

4. Control

4.1. Introduction

In the past, control of GI-nematode infections was often restricted to FSG calves, as these animals are the most susceptible to clinical disease. Over the last decades several anthelminthic control programmes have been developed for use in FSG calves. In general, these control programmes are based on treatments during the early part of the first grazing season to prevent recycling of the infection acquired from the over-wintered larvae on pasture. Well-known examples are the ivermectine 0-6, doramectine 0-8 or fenbendazole bolus systems (e.g. Eysker *et al.*, 1998; Fisher *et al.*, 1995; Bauer *et al.*, 1997). More recently, the use of anthelmintic drugs in second and later grazing seasons has increased (Ploeger *et al.*, 2000), promoted by the evidence of production benefits after anthelmintic use in older animals (Hawkins, 1993; Sanchez *et al.*, 2004a) and the development of anthelmintics with a zero-withdrawal time for milk (e.g. Shoop *et al.*, 1996b). In the following paragraphs, the anthelmintics and systems to control GI-nematode infections in adult dairy cows will be discussed.

4.2. Anthelmintics

There is a great repertory of drugs that can be used for the control of GI-nematode infections in cattle. The anthelmintics that are registered in Belgium for use against GI nematodes in cattle are shown in Table 1.3. The latest class of anthelmintics that was introduced to the market are the macrocyclic lactones (MLs). MLs outperform the older anthelmintics by their higher efficacy and safety levels and they are now widely used for

the control of nematode infections in cattle. The main mode of action of MLs is the activation of glutamate-gated chloride channels that are found on the membranes of the pharynx and particular neurones of the nematode. As a result, MLs stimulate an influx of chloride ions into the cells, resulting in paralysis and death of the parasite (Martin *et al.*, 2002). MLs have a broad-spectrum activity against both endo- and ectoparasites and are therefore also called endectocides. There are only 2 ML-products that are registered for use in lactating dairy cattle: pour-on formulations of eprinomectin and (in some countries) moxidectin. The pharmacokinetic and milk-residue profiles in lactating animals have especially been documented for eprinomectin. Eprinomectin was discovered by testing hundreds of ML-analogues and selecting the molecule that had the lowest proclivity to partition in the milk (Shoop *et al.*, 1996a). Subsequently, Alvinerie *et al.* (1999) examined the pharmacokinetics of eprinomectin in lactating cattle after topical delivery. They stated that the effect of a drug is related to the systemic area under the curve (AUC). The observed area under the plasma concentration curve for eprinomectin (239 ng/day ml) was greater than the values of ivermectin (115 ng/day ml) or doramectin (168 ng/day ml) after topical delivery, observed by Gayrard *et al.* (1999). The larger AUC observed for eprinomectin may result from a higher bioavailability of the eprinomectin pour-on formulation. The maximum plasma concentration (C_{max}= 43.8 ng/ml) was reached 2 days post administration. Finally, it was observed that only approximately 0.1 % of the total administered dose was eliminated in the milk, which is 50-fold less than for either ivermectin or moxidectin after subcutaneous administration. The maximum level of residue in milk did not exceed the maximum acceptable limit of 30 ng/ml (Alvinerie *et al.*, 1996; 1999).

The efficacies of topically administered eprinomectin or moxidectin are highly comparable. After experimental infection of calves with all of the major GI and lung nematodes, eprinomectin pour-on (0.5 mg/kg) removed ≥ 99 % of the adult and larval stages of every species (Shoop *et al.*, 1996b). The high activity of eprinomectin against most GI-nematode species was confirmed in several field trials in different geographical locations where efficacies of > 99 % were observed against all nematode species for which moderate to high burdens occurred in the untreated

controls (e.g. Gogolewski *et al.*, 1997; Pitt *et al.*, 1997; Williams *et al.*, 1997). The persistent efficacy of eprinomectin was investigated by 6 studies on 159 calves (Cramer *et al.*, 2000). The authors found ≥90 % efficacy against challenge infections with *Haemonchus placei, Trichostrongylus axei and T. colubriformis* at 21 days after treatment and against *O. ostertagi, C. oncophora, D. viviparus, Nematodirus helvetianus* and *Oesophagostomum radiatum* at 28 days after treatment. Moxidectin pour-on (0.5 mg/kg) removed > 99 % of adult and larval nematode species in naturally infected calves and lactating dairy cows (Morin *et al.*, 1996). The high efficacy of moxidectin was confirmed in lactating dairy cows where an overall efficacy of 99 % was observed (Yazwinsky *et al.*, 1999). A persistent efficacy > 90 % has been recorded for moxidectin pour-on for more than 14 days against *C. oncophora* and > 99 % for 35 to 42 days against *O. ostertagi* and *D. viviparus* (Eysker and Eilers, 1995; Hubert *et al.*, 1997; Vercruysse *et al.*, 1997).

Although it appears that also after subcutaneous administration eprinomectin has a very low milk / plasma partitioning (Baoliang *et al.*, 2006), eprinomectin has only been commercially developed for topical delivery. This method is consumer-friendly and it was demonstrated that optimal efficacies were maintained when eprinomectin was administered to various hair coats (short/long) and under a wide range of weather conditions (Gogolewski *et al.*, 1997). A drawback is that topical delivery results in a higher-variable systemic availability. After topical delivery of eprinomectin, large between-animal variations in C_{max} were observed (Alvinerie *et al.*, 1999). Similar variability has also been observed for ivermectin and doramectin (Gayrard *et al.*, 1999).

Later, it was demonstrated that the natural licking behaviour of cattle has a marked influence on the systemic availability of endectocides after pour-on administration. Prevention of self- and allo-licking resulted in a lower and less variable systemic availability of ivermectin (Laffont *et al.*, 2001).

Table 1.3. Anthelmintic drugs registered in Belgium for use in cattle against gastrointestinal nematodes. Products with combinations of different drugs are not enlisted

Anthelmintic drug	Formulation	Withdrawal time (days)	
		Meat	Milk
Benzimidazols and pro-benzimidazols			
Albendazol	Suspension 10 %	14	4
Febantel	Pellets 1.9 %	14	4
	Granules 10 %	14	4
Fenbendazol	Suspension 10 %	14	4
	Granules 22 %	14	4
	Bolus 12 g	200	N.R.
	Fodder block	14	N.R.
Netobimin	Drench 10 %	10	2
Oxfendazol	Drench 5 %	10	2
	Bolus 3.75g	180	180
	Bolus 6.25 g	180	180
Imidathiazols			
Levamisol	Bolus 22.05 g	112	N.R.
	Inj. Solution 10 %	28	N.R.
	Inj. Solution 7.5 %	7	N.R.
	Pour-on 20 %	3	N.R.
Macrocyclic lactones			
Doramectin	Inj. Solution 1 %	42	N.R.
	Pour-on 0.5 %	35	N.R.
Eprinomectin	Pour-on 0.5 %	15	0
Ivermectin	Inj. Solution 1 %	21 – 42[a]	N.R.
	Pour-on 0.5 %	17 – 28[a]	N.R.
Moxidectin	Inj. Solution 1 %	65	N.R.
	Inj. Solution 10 %	108	N.R.
	Pour-on 0.5 %	14	0

(a: Withdrawal time differs between different commercial products; N.R.: not for use in cows from which milk is destined for human consumption)

In cows that were allowed to exhibit their natural licking behaviour, it was calculated that 58-78 % of the ivermectin pour-on dose was ingested, while only 10 % was absorbed percutaneously. Approximately 72 % of the ingested dose transited directly into the faeces (Laffont *et al.*, 2003). Sallovitz *et al.* (2005) observed that the doramectin-concentration profiles in the mucosal tissue and luminal contents of the abomasum and duodenum were markedly higher in free-licking calves than in non-licking

calves. Finally, it was shown that 2 non-treated cows ingested 22 and 27 % of a pour-on dose by allo-licking when they were grazed together with 6 cows that received a topical treatment (Bousquet-Mélou *et al.*, 2004). These studies suggest that pour-on formulations of an anthelmintic are most suitable for whole-flock treatments because treating only a small proportion of a flock may result in a decreased efficacy against the parasites. For anthelmintic trials these results imply that pour-on formulations cannot be used for treating one half of a herd using the other half as control group, unless the 2 groups can be separated for several days.

4.3. Control methods

4.3.1. Anthelmintic treatments

The impact of anthelmintic treatment on milk production has been studied since the 1970's. In spite of this, uniform recommendations for anthelmintic-treatment strategies in adult dairy cows have never been developed. A key problem to do so was the unpredictability of the production response after treatment (Vercruysse and Rew, 2002). However, based on the data of recent studies a few recommendations could be proposed. Studies evaluating the effect of MLs have greater production responses and less variable results than studies using older anthelmintics (Gross *et al.*, 1999; Sanchez *et al.*, 2004a) and MLs with a zero-withdrawal time for milk (eprinomectin, moxidectin) can therefore be considered as the drugs of choice. Another important issue was the best timing of treatment. In the meta-analysis of Sanchez *et al.* (2004a), trials which applied the treatment in mid-lactation or strategically throughout the year had higher production responses (+ 0.4 kg/cow per day) compared with trials treating at dry off or at calving. However in recent trials, good results were equally obtained after eprinomectin treatment at calving (Nødvedt *et al.*, 2002; Sanchez *et al.*, 2005). The effects were independent of calving season, although the power of the studies to detect such an effect might have been insufficient (Nødvedt *et al.*, 2002). In a study in the UK on 24 spring-calving multiparous cows, half of the cows received 3 treatments with eprinomectin across lactation at 7-week

intervals, starting 1 month after turnout (Gibb *et al.*, 2005). Significant increases in the mean daily yield were observed following each of the 3 treatments, indicating a positive response to repeated treatments at different stages of lactation. Consequently, the timing of treatment seems less critical than previously thought and it seems that it can be chosen according to the farm-specific objectives and management system.

Over the last years, there are increasing reports on cattle nematodes that have developed resistance against MLs (e.g. Mejia *et al.*, 2003; Loveridge *et al.*, 2003). The most important issue in the development of anthelmintic resistance is the contribution that those nematodes surviving therapy make to the next generation, which in turn depends on the proportion of a parasite population that escapes exposure to control measures (*refugia*) (van Wijk, 2001). This understanding has led to the proposal that control schemes should keep a substantial proportion of the parasite population in refugia by targeting anthelmintic treatments to those members of a flock or herd showing clinical symptoms or a reduced productivity due to nematode infection (van Wijk *et al.*, 2006). However, in cows the lack of good biomarkers has thus far prevented the development of such selective treatment programmes. The age and production level of the cows seem to be no good biomarkers to indicate which cows would respond with the highest production increase to anthelmintic treatment. Two studies report different (but contradictory) effects between primiparous and multiparous cows (McPherson *et al.*, 2001; Forbes *et al.*, 2004), but other studies could not find an age-dependent effect (Ploeger *et al.*, 1989; Nødvedt *et al.*, 2002). Studies of Ploeger *et al.* (1989; 1990c) indicate that high producing cows had a greater milk-yield increase than low producing cows. However, in a more recent study no such production-level dependent effect was observed (Nødvedt *et al.*, 2002). The only promising indicator to predict the production benefits after treatment seems the *Ostertagia*-specific antibody level (see diagnosis).

4.3.2. Non-chemotherapeutic control options

Due to the increasing concern about the development of resistance of GI nematodes against the available anthelmintics, non-chemotherapeutical

control options will probably play an increasing role in the future (Jackson and Miller, 2006). Of the non-chemotherapeutic control options for cattle, grazing management is the only option that can be directly applied on the farms. Control by grazing management aims at limiting the contact between host and parasite. Practices that have shown to be effective in the control of GI nematodes are late turnout, movement to aftermaths, well-designed rotational grazing systems, and alternate/mixed grazing of different host species and cow-calf pair grazing (Nansen *et al.*, 1987; Barger, 1997). However, grazing management practices have mostly been evaluated for the control of GI nematodes in FSG calves (e.g. Hansen *et al.*, 1989; Shaw *et al.*, 1998a,b) and their use for nematode control in adult cows has received little attention. A Canadian study reported an increased exposure to GI nematodes as measured by anti-*O. ostertagi* antibody levels in bulk milk when milking cows or heifers had a greater exposure to pasture, when manure was spread onto the pasture and when heifers were allowed to graze the same pastures as dry cows (Guitián *et al.*, 2000). Two other studies confirmed the effect of amount of exposure to pasture of cows, but could not confirm the associations with spreading manure or grazing together of heifers and cows. In addition, variable results were found for factors such as anthelmintic treatment, pasture rotation and mowing (Caldwell *et al.*, 2002; Sanchez and Dohoo, 2002). In a trial in New-Zealand, the milk production increase after anthelmintic treatment was greater on farms where calves had received a minimal number of drench treatments than on farms where calves had received regular treatments (Bisset *et al.*, 1987). The results suggested that the grazing system employed was of importance in so far as it determined the level of exposure of cows to calf-contaminated pasture.

Other non-chemotherapeutic control options are genetic selection for resistance against GI nematodes, vaccination, nematophagous fungi, dietary supplementation with protein and grazing of plants containing anthelmintic substances such as condensed tannins (Jackson and Miller, 2006; Waller, 2006). However, none of these options have yet reached the stage of commercialisation in cattle due to the lower efficacies and more variable results in the control of nematode infections than synthetic drugs. Moreover, these options are currently only being evaluated in FSG

calves and their effects in adult cattle remain unstudied (Jackson and Miller, 2006).

5. Conclusions

GI nematodes can have a negative impact on the productivity of adult dairy cows. The results from anthelmintic treatment trials indicate that at least in some herds anthelmintic treatment of the adult cows is economically profitable. The major problem remains how to monitor GI-nematode infections in an adult dairy herd to indicate if the infection is causing a loss in productivity. *Ostertagia*-specific antibody level determination is the most promising method for this purpose, but more data are required to investigate its usefulness. In particular, more knowledge is required on the test characteristics of the ELISA, the ability of the test to monitor the effects of control measures and the predictive value of the test on the production response after anthelmintic treatment.

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OBJECTIVES

The literature review (chapter 1) highlights the potential of *Ostertagia ostertagi*-specific antibody levels in milk to study GI-nematode infections in dairy cows. However, knowledge is missing on how this parameter can be used for herd-health monitoring purposes.

Therefore, the overall objective of this thesis is to study the epidemiology and impact on production of GI-nematode infections in dairy cows by the use of anti-*O. ostertagi* antibodies in milk.

The sub-objectives are:

- To develop and validate an ELISA that detects and quantifies *Ostertagia*-specific antibodies in milk (chapter 2 and 3).
- To investigate the associations between *Ostertagia*-specific antibody levels in bulk-tank milk and milk-production parameters (chapter 4).
- To investigate the associations between *Ostertagia*-specific antibody levels in bulk-tank milk and herd-management factors (chapter 5).
- To investigate the value of *Ostertagia*-specific antibody levels in bulk-tank milk to predict the milk-yield response after anthelmintic treatment (chapter 6).

CHAPTER 2

Assessment of the repeatability of an *Ostertagia ostertagi* milk ELISA and effects of sample preparation*

*Based on the manuscript: Charlier, J., Duchateau, L., Claerebout, E., Vercruysse, J., 2005. Assessment of the repeatability of a milk *Ostertagia ostertagi* ELISA and effects of sample preparation. Prev. Vet. Med. 68, 277-288.

1. Introduction

Although GI-nematode infections in adult cows are usually subclinical, they have been associated with decreased levels of milk production (Gross *et al.*, 1999; Sanchez *et al.*, 2004). The major problem however remains to identify the herds where the infection level is high enough to justify an anthelmintic treatment (Vercruysse and Claerebout, 2001). Diagnostic techniques such as FECs and serum pepsinogen assays have been shown to be of limited use in adult dairy cattle (Ploeger *et al.*, 1989, 1990; Berghen *et al.*, 1993). Currently, detection of anti-*Ostertagia ostertagi* antibody levels is considered as a very promising method (Eysker and Ploeger, 2000; Sanchez *et al.*, 2002a). *Ostertagia*-specific antibody levels can be detected and quantified by an ELISA and especially their determination in bulk-tank milk would be suitable for a regular monitoring of the parasitic infection level of a herd.

However, a good interpretation of test results is impossible without knowledge of the test characteristics. Previously, the repeatability of the test was evaluated without discriminating between serum and milk samples. Two different graphical tools, the concordance correlation coefficient (CCC) plot (Lin, 1989) and the Bland-Altman plot (Bland and Altman, 1986) were used for this purpose (Sanchez *et al.*, 2002b). Both plots indicate to what extent 2 replicates of a set of samples have similar values. In both plots 1 replicate is compared with another. It is obvious that the large amount of replication used in this validation study leads to an even larger number of plots as relationships can only be represented pairwise. Therefore, in this paper a new graphical method is proposed to assess the variability of several measurements of a milk *O. ostertagi* ELISA on the same sample over time. Furthermore the effect of different sample preparations on the test results is investigated.

2. Materials and methods

2.1. Milk samples

A total of 82 milk samples were collected at 42 dairy herds in Flanders by convenience sampling. The samples consisted out of 42 bulk-tank milk

samples from an equal number of herds and 40 individual milk samples from 2 herds.

After collection, the samples were handled following a standard procedure. The samples were centrifuged (16,000 *g* for 5 min), the fat was removed and the underlying supernatant was collected and frozen (-20 °C). All this was done on the day of sample collection. To remove all fat, the samples were thawed and recentrifuged (16,000 *g* for 5 min) before testing. This standard procedure was modified for the samples that were used to study the effect of different sample preparations on the test results.

2.2. ELISA

Crude antigen of adult *O. ostertagi* worms was prepared as described by Keus *et al.* (1981). The samples were tested using a crude-antigen *O. ostertagi* ELISA as described by Sanchez *et al.* (2002b), with modifications. Flat bottom, 96 well microplates (Nunc Maxisorp) were coated with the antigen at a concentration of 1 μg/ml in a 0.050 M carbonate-bicarbonate buffer (pH 9.6) and incubated overnight at 4 °C. Wells were washed 3 times with 0.3 ml PBST (phosphate-buffered saline with 0.05 % Tween-20). Non-specific binding sites were blocked by adding 200 μl per well of 3 % fetal calf serum (Gibco BRL, Life Technologies) in PBST. Plates were incubated for 1 h at 20 °C and washed as before. Milk samples (100 μl) were added undiluted to the wells. On each plate a negative and a positive control milk sample were included in 6 wells each. The negative control sample was a bulk milk sample from a herd where the adult dairy cows had no access to pasture. The positive control sample was a bulk milk sample of a highly infected herd (based on herd anamnesis and previous worm counts). After incubation and washing as before, rabbit anti-bovine IgG coupled to horseradish peroxidase (Jackson ImmunoResearch Laboratories) (1/1500 in PBST/1 % fetal calf serum) was added as conjugate. Plates were incubated and washed as before. ABTS substrate (50 mg) (2,2'-azino-bis-3-ethylbenzothiazoline-sulfonic acid) (ABTS tablets, Boehringer Mannheim) was diluted in 50 ml of freshly prepared buffer (ABTS buffer, Boehringher Mannheim). After incubation for 30 min in the dark at 20 °C, absorbance was read at 405

nm and at 492 nm. The optical densities were expressed as a ratio (ODR) following the formula ODR= (OD – NC) / (PC – NC), where OD is the result of the subtraction of the OD at 492 nm from the OD at 405 nm and NC and PC are the ODs of the negative and positive control respectively.

2.3. Evaluation studies

2.3.1. Border-effects trial

To evaluate the uniformity of the test results within a plate, 2 plates were tested. Each plate contained an identical milk sample in all its wells (except for 4 wells of the second plate, which contained conjugate and substrate controls). A different milk sample was used for the 2 plates. Border effects were studied.

2.3.2. Repeatability trial

Forty milk samples were tested in duplicate per plate on 4 different ELISA plates per day. This was repeated during 3 days. Out of this test the between-duplicate, between-plate and between-day repeatability were determined and compared to the between-sample variability.

2.3.3. Effect of different sample preparations

To study the effect of different sample preparations on the test results, 40 milk samples were each divided into 10 aliquots. A different preparation was tested in each aliquot (Table 2.1). Each ELISA plate contained 8 samples. The samples were systematically ordered on a plate according to a latin-square design plan: each row contained 10 aliquots of 1 sample. The control samples were added in the border columns. The 10 aliquots were added in the 10 centre columns of the plate in a fixed order, each row beginning with the 2nd aliquot of the previous row. In total 40 samples were tested on 5 different ELISA plates. This experiment was repeated once.

Table 2.1. Different sample preparation techniques that were investigated in this study to determine the effect of sample storage and processing on ODR values

Aliquot	Factor
1	standard procedure (storage for 1 day at 4 °C)
2	1 extra freeze-thaw cycle
3	2 extra freeze-thaw cycles
4	samples centrifuged only once (after collection, and than stored at –20 °C)
5	samples centrifuged only once (after storage at –20 °C)
6	whole milk, cream border is pricked
7	whole milk, samples are shaken
8	storage for 2 days at 4 °C
9	storage for 3 days at 4 °C
10	storage for 4 days at 4 °C

2.4. Statistical analysis

The data of the border-effects trial were analysed by comparing the OD readings of the inner wells (separated at least 2 wells from the border) with the OD readings of the outer wells (wells at the border of the plate) separately for each of the 2 plates by a 2-sided t-test.

The test repeatability based on the ODR values was evaluated by 2 methods. In the first method, an alternative graphical method to assess repeatability within a series of replicates over different days was developed. In order to assess whether repeatability within plates changed over time, first the within-plate residuals $\left(Y_{ijkl} - \overline{Y}_{ijk.}\right)$ were derived, with $\overline{Y}_{ijk.}$ the mean of the 2 observations on the same sample *i* within plate *k* on the same day *j*. These within-plate residuals were plotted sequentially according to test day and within test day according to sequence number of the plate. Within a plate the residuals were plotted per column from upside down, shifting from the left to the right side of the plate. Plates characterized by a higher variability can be spotted immediately on these plots, as the residuals are deviating much more from 0. In order to assess repeatability over plates, the between-plate residuals $\left(\overline{Y}_{ijk.} - \overline{Y}_{i...}\right)$ were derived, with $\overline{Y}_{i...}$ the mean for sample *i* over the different plates. Again, these between-plate residuals were plotted sequentially, and plates that differed substantially from other plates can be spotted immediately.

In the second method, the test repeatability over duplicates within plate, plates and days was modelled by the following random-effects model:

$$Y_{ijkl} = \mu + s_i + d_j + p_{k(j)} + e_{ijkl}$$

with Y_{ijkl} the l^{th} measurement of sample *i* on plate *k* on day *j* (*l*= 1,2; *i*= 1-40; *k*= 1-4; *j*= 1,2), μ is the overall mean, s_i is the effect of sample *i* ~ N(0, σ_s^2), d_j is the effect of day *j* ~ N(0, σ_d^2), $p_{k(j)}$ is the effect of plate *k* within day *j* ~ N(0, σ_p^2) and e_{ijkl} is the random-error term ~ N(0, σ^2).

Interaction terms were evaluated but not included in the model as they were not significant. Only the ODR values of the first 2 days were used in this analysis. The variance components related to the different sources of variability were estimated by restricted maximum likelihood (Patterson and Thompson, 1971). Normality of the observed ODR values and the residuals was investigated by a histogram (Fig. 2.1) and a normal-probability plot.

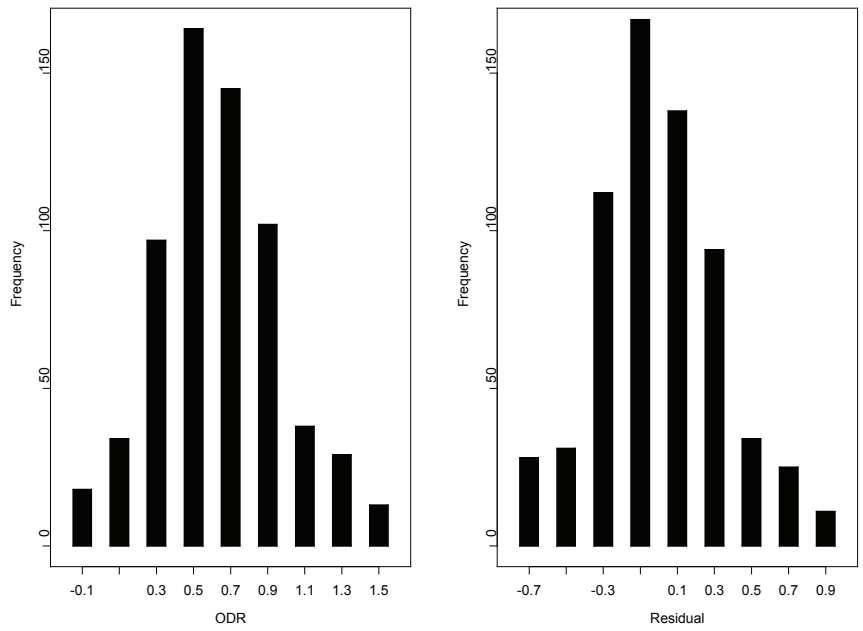

Fig. 2.1. Histograms of the observed ODR values and the residuals of the random-effects model in the trial to investigate the repeatability over replicates, plates and days.

Based on these diagnostics, there was no reason to reject the normal-distribution assumption. Based on the variance-component estimates, the amount of variance that could be attributed to each source of variability (replicate, plate, day) was expressed as a proportion of the total between-sample variance. Overall measures with respect to duplicate within plate, plate and day variability were obtained as follows. The variance of the

difference between 2 measurements from the same sample and same plate is given by $\mathrm{Var}\left(Y_{ijkl} - Y_{ijkl'}\right) = 2\sigma^2$, the variance of the difference between 2 measurements from the same sample and different plates on the same day is given by $\mathrm{Var}\left(Y_{ijkl} - Y_{ijk'l'}\right) = 2\left(\sigma^2 + \sigma_p^2\right)$, the variance of the difference between 2 measurements from the same sample but on a different day is given by $\mathrm{Var}\left(Y_{ijkl} - Y_{ij'k'l'}\right) = 2\left(\sigma^2 + \sigma_p^2 + \sigma_d^2\right)$, and finally the variance of the difference between 2 measurements from a different sample on a different day is given by $\mathrm{Var}\left(Y_{ijkl} - Y_{i'j'k'l'}\right) = 2\left(\sigma^2 + \sigma_p^2 + \sigma_d^2 + \sigma_s^2\right)$. Finally, the different sample preparation techniques were evaluated by a mixed model with day, plate, row and column as random-effect factors and the sample-preparation technique as a fixed-effect factor. The 95 % confidence intervals (CI) for the difference between the alternative techniques and the standard technique were derived in order to evaluate equivalence.

3. Results

3.1. Border effects

The OD readings of the inner wells were higher than those of the outer wells. A mean difference of 0.067 ($P< 0.001$) and 0.098 ($P= 0.016$) was observed both in plate 1 and plate 2 respectively. The 2 ELISA plates are shown in Fig. 2.2.

3.2. Repeatability over replicates, plates and days

The alternative graphical procedure is demonstrated in Fig. 2.3. The within-plate residuals are approximately the same for all the tested plates and most of the observations lie between –0.1 and 0.1. Plate 6 has a lower and plate 8 a higher variability than the other plates. When the between-plate residuals are observed, a deviating pattern is obvious on all the plates of the third day. While most of the residuals lie between –0.2 and 0.2 on the first and second day, they range between –0.6 and 0.2 on the third day. Moreover, the plot reveals that on the third day there is a trend of increasing negative between-plate residuals for samples that

were tested on the right side of the plate. On this day abnormal values of the positive control were seen, with values on the same plate ranging between 0.484 and 1.150.

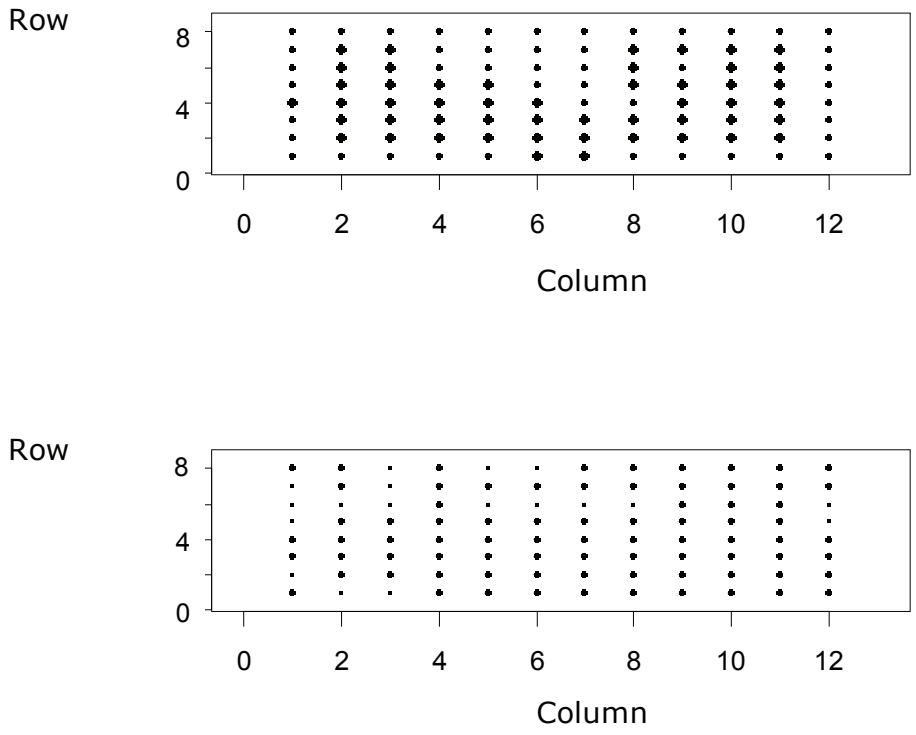

Fig. 2.2. Two ELISA plates, each containing an identical milk sample in all their wells. A different sample was used for the 2 plates. The second plate contained 2 conjugate (row 5, column 1 and 12) and 2 substrate controls (row 6, column 1 and 12). The diameter of the dots represents the ODR values.

These results were confirmed by the CCC and Bland-Altman plots. The repeatability between day 1 and day 2 was high. The mean CCC was 0.96 with a range of 0.93 to 0.98. The mean limits of agreement were –0.19 to 0.13 with a mean difference of –0.03. The results of the third day showed a low repeatability. When the results of the first and second day were compared with the results of the third day the mean CCC was 0.68 and the mean limits of agreement were –0.18 to 0.57 with a mean difference of 0.18. Fig. 2.4 shows an example of CCC measurements and Bland-Altman-plots where the ODR readings of 3 plates that were tested on 3 different days are compared.

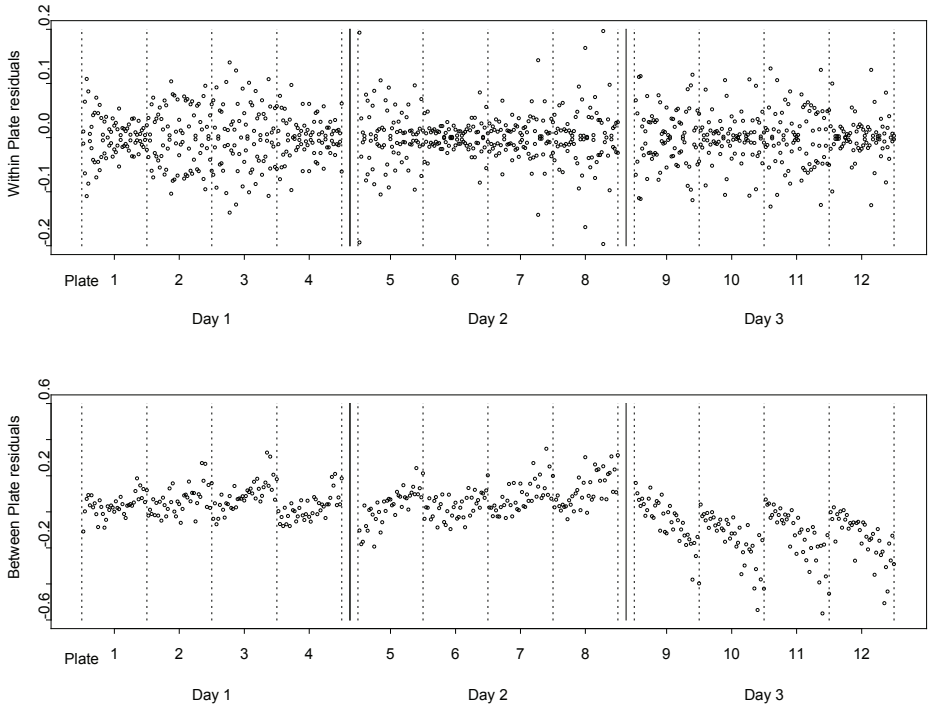

Fig. 2.3. The within-plate residuals and between-plate residuals plotted sequentially per plate and per day.

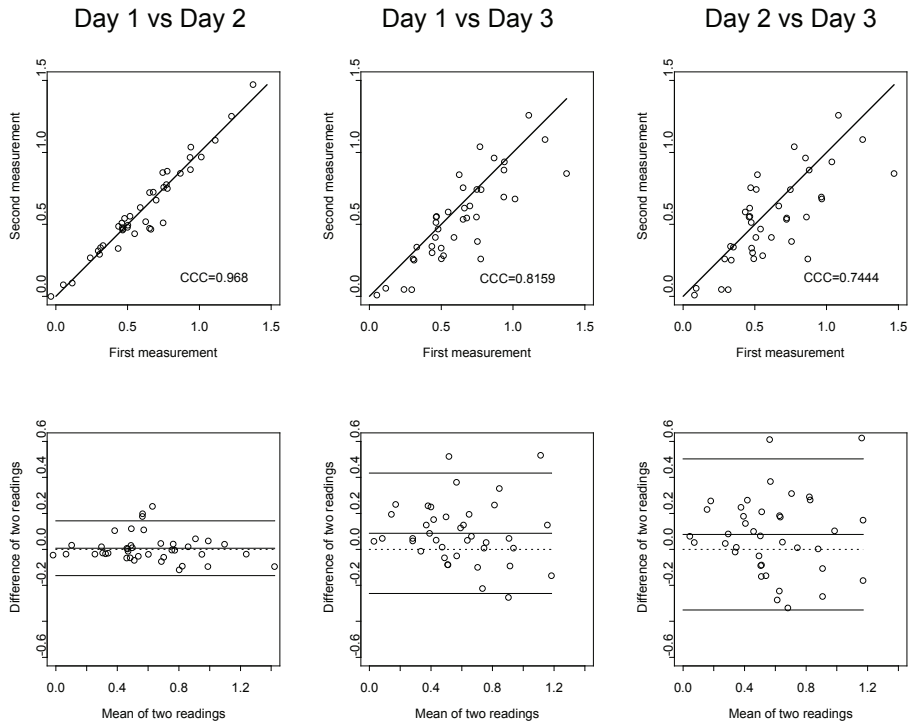

Fig. 2.4. CCC and Bland-Altman plots of 40 milk samples that were tested on different days. Every plot represents the comparison between 2 ELISA plates.

The random-effects analysis resulted in a total-variance estimate of 0.113. Ninety-four percent of the total variance was attributable to the milk sample, 5 % to the duplicates within a plate, 1 % to the plate within the same day and 0 % to the day. In other words, 94 % of the variability was explained by the milk sample and 6 % was explained by the assay variability. Based on these estimates, the overall variability of the difference between 2 measurements as a function of their origin (replicate, plate and day) was deduced. The expected 95 % range is -0.14 to 0.14 for different ODR readings of the same sample on the same plate and -0.16 to 0.16 for different ODR readings of the same sample on different plates or on different days.

3.3. Effect of sample preparation

The pairwise differences between the standard procedure of sample preparation and the alternative sample preparations are presented in Fig. 2.5. None of the alternative sample preparations had a significant influence on the ODR readings. The largest difference with the standard procedure was found for storage for 2 days at 4 °C and is estimated as 0.025 with a CI of [–0.004;0.053]. Consequently, we can exclude that on average the difference between the standard procedure and this treatment is larger than 0.053 with 95 % confidence.

4. Discussion

While this study focuses on test characteristics as repeatability and robustness, there are only few studies that have investigated the species specificity of the antigen. Cross-reactions with *Cooperia* spp. have been demonstrated (Keus *et al.*, 1981) but are not considered as a disadvantage since the ELISA is used to measure total GI-parasite load, rather than just *O. ostertagi* infections. Cross-reactions with other helminths like *Fasciola hepatica* may occur (Eysker and Ploeger, 2000).

The border-effects trial revealed that the outer wells had significantly lower OD values than the inner wells. This observation may be explained by an "edge effect" and is caused by the outer wells being more influenced by temperature in the binding reactions than the inner wells

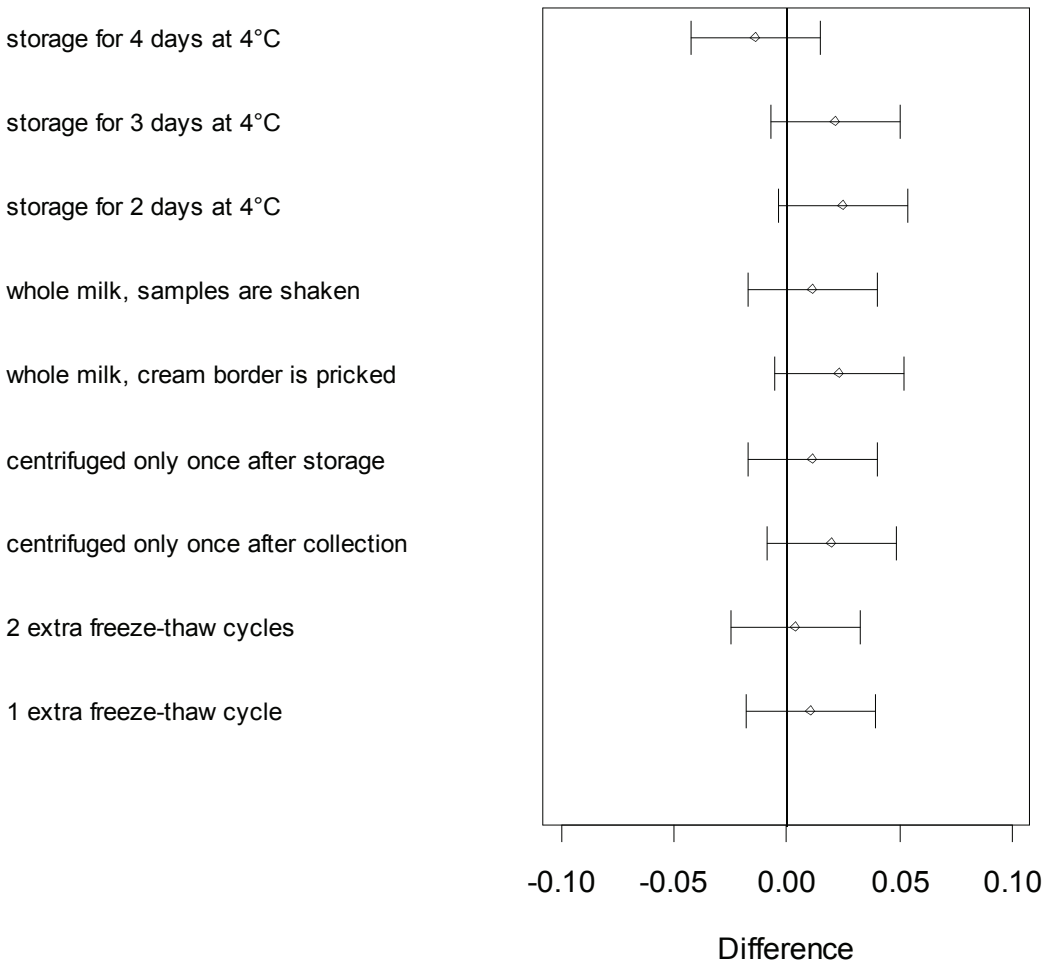

Fig. 2.5. The pairwise differences between the standard procedure of sample preparation and the different sample preparations with their 95 % confidence intervals.

(Venkatesan and Wakelin, 1993). This edge effect can be reduced by not using the outer wells of the ELISA plate. In order to control for this effect, in the study to assess the effect of different sample treatments, the row was used as a blocking factor (each sample was tested in 1 row).

Sanchez *et al.* (2002b) demonstrated previously that adjusting the raw ODs to ODRs gave the most repeatable results for the studied ELISA. Since the ODR values are the ones that are used in practice, in the repeatability trial only the variability that is left after normalization to ODR values was investigated.

The alternative graphical procedure based on within and between plate residuals was very useful in the detection of abnormal ELISA plates. The

plates that were tested on the third day were detected as "abnormal" and were therefore not included in the random-effects model. It was observed that the control samples had abnormal values on these plates. Previously, Sanchez *et al.* (2002b) reported a low repeatability of the ELISA when the control samples were higher than expected. Therefore, in practice the control samples can be used to decide when a certain plate has to be rejected. For instance, control charts can be used to detect the plates where the positive or negative control deviates more than 2 standard deviations from 30 preliminary observations (Levey and Jennings, 1992).

The results of the graphical procedure based on within- and between-plate residuals are confirmed by the CCC and Bland-Altman plots. However, a drawback of these methods is that they did not allow to investigate the results in 1 plot or to easily detect plate-related problems.

The random-effect analysis allows to investigate different sources of variability and to assess the additional variability that is caused by each source of variation that is added in the model. According to Fleiss (1986), the amount of variability that was explained by the milk sample and the assay variability indicates an excellent repeatability of the ELISA. The results demonstrate that deviations in the ODR readings of –0.16 to 0.16 are observed when a sample is repeatedly tested over different plates. No additional between-day variability was observed. However this result has to be used with much caution since only 2 days were investigated. The between-duplicate variability was responsible for a considerable amount of additional variability. Therefore it is useful to test each sample in duplicate.

Since no significant effects were found for 1 or 2 extra freeze-thaw cycles, the samples can be frozen and retested on another day. Sanchez *et al.* (2002b) reported that milk samples that were preserved with bronopol could be stored up to 42 days at 4 °C, without seriously affecting the test results. Also in this study, where no bronopol was added to the samples, the milk samples could be stored for at least 4 days at 4 °C, without affecting the test results. However, there was a trend to lower ODR values when the samples were stored for 4 days (Fig. 2.5) and consequently it is not recommended to store samples longer than this period before testing. Other studies reported that bovine or human IgG content in milk is not

changed during conservation at 4 °C during 24h or 72h (Li-Chan *et al.*, 1995; Lawrence, 1999). Taking into account that fat could interact with the quantification of milk Igs, most parasitological milk ELISAs are performed on skimmed milk (e.g. Boulard *et al.*, 1985; Otranto *et al.*, 2001; Chanlun *et al.*, 2002). However, the centrifugation and collection of lactoserum is a time consuming procedure and can be a restriction when the ELISA is used on broad scale or when it would be automatized. In the present study, no significant difference between skimmed and non-skimmed milk samples was observed. The results agree with previous studies where no interaction between milk-fat and bovine-IgG determination was found (Fleenor and Stott, 1981; Mainer *et al.*, 2000). This finding has the implication that the test can be used in large-scale studies and should encourage research for automatization of the ELISA so that it can really become a standard-monitoring tool in bovine medicine.

5. Conclusion

The milk *O. ostertagi* ELISA has a good repeatability. However, a decision should be made for each plate if it can be accepted based on the values of the control samples. An edge effect can occur and therefore the variability can be reduced by not using the outer wells of the ELISA plate. The ELISA is a robust technique concerning sample storage and sample preparation and the technique can be simplified by omitting skimming of the milk samples. The proposed alternative graphical procedure based on within- and between-plate residuals proved to be useful in the detection of abnormal ELISA plates and can be used in the evaluation of other quantitative ELISAs.

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CHAPTER 3

The effect of an experimentally induced acute mastitis on the test results of an *Ostertagia ostertagi* milk ELISA*

* Based on the manuscript: Charlier, J., Duchateau, L., Vangroenweghe, F., Claerebout, E., Burvenich, C., Vercruysse, J., 2006. The effect of an experimentally induced acute mastitis on the test results of an *Ostertagia ostertagi* milk ELISA. Vet. Parasitol. 136, 161-165.

1. Introduction

Antibody levels against *Ostertagia ostertagi* in the milk are considered to be a promising parameter to identify dairy cows or herds with production losses due to GI nematodes (Sanchez *et al.*, 2002). The antibody levels are measured with an indirect ELISA. Especially the determination of these antibodies in bulk-tank milk would be an easy and cost-effective way to monitor the GI-nematode infection status of a herd. It was demonstrated that the serum antibody level is by far the most influential factor in determination of the *O. ostertagi* milk antibody level (Kloosterman *et al.*, 1993). Other factors such as milk yield, age, stage of lactation and sire effects can also influence the milk antibody levels, but to a lesser extent (Kloosterman *et al.*, 1993; Morris *et al.*, 2002). In addition, Sanchez *et al.* (2004) found a positive correlation between the specific milk antibody levels and somatic-cell count, indicating that mastitis could affect the test results. Since the total IgG concentration is approximately 35 times higher in serum than in milk (Butler, 1986), we hypothesized that an acute mastitis could cause a flow of specific and non-specific antibodies from serum to the milk and that the test results could be affected considerably. Therefore, the objectives of this study were (1) to investigate the effect of an induced acute mastitis on the test results and (2) to estimate the effect of the contribution of milk from 1 or more infected quarters on the antibody levels in bulk-tank milk.

2. Materials and methods

2.1. Experimental animals and study facilities

Twenty-five primiparous cows were used in this study. The cows had grazed and were considered infected with GI nematodes. This was confirmed by the presence of nematode eggs in the faeces of 15 animals. The animals were free of major mastitis pathogens, which was confirmed through 3 consecutive bacteriologically negative examinations. At the time of *Escherichia coli* inoculation, the animals were between 12 and 28 days post-parturition. The experimental infections were approved by the Ethical

Committee of the Faculty of Veterinary Medicine (Ghent University, Merelbeke, Belgium).

2.2. Intramammary Inoculation Procedure

The cows were inoculated in their left quarters with 1 x 10^4 colony-forming units *E. coli* P4:O32 as described by Hoeben *et al.* (2000). Following inoculation, a control sample of the inoculum was checked for correct preparation and dose administration as described by Vangroenweghe *et al.* (2005).

2.3. Sample collection

Quarter milk samples were collected manually at –24 h, 0 h, 12 h, 24 h, 72 h and 144 h after infection. Milk samples for the *O. ostertagi* ELISA, IgG ELISA and for the determination of sodium and chlorine concentration were centrifuged to remove the fat, the underlying supernatans was collected and frozen at –20 °C until analysis. The milk samples for determination of somatic-cell count were sent to the Milk Control Centre (MCC) Flanders (Lier, Belgium) for further analysis.

2.4. Laboratory methods

Antibody levels against *O. ostertagi* were determined with an ELISA as previously described in chapter 2 and the test results were expressed as an ODR.

Total IgG levels were measured using a commercial ELISA kit (bovine IgG ELISA quantitation kit, Bethyl Laboratories, Montgomery, TX, USA) according to the manufacturer's recommendations. Milk samples (100 µl) were tested in a dilution of 1/25,000. The IgG levels were expressed as ODR= (OD sample – LS) / (HS - LS), where LS and HS are the ODs of a low (7.8 ng/ml) and high (500 ng/ml) concentration reference serum sample, respectively.

The sodium and chlorine concentration (mmol/l) were used as measures for the damage to the blood-milk barrier (Burvenich, 1983) and were determined by an ion-selective electrode analyzer (Ilyte®; Instrumentation Laboratories, Milan, Italy). The somatic-cell count was determined using a

fluoro-opto electronic method (Fossomatic 5000 cell counter; Foss Electrics, Hillerød, Denmark) by the MCC Flanders.

2.5. Titration experiment

From 17 animals, a sample from a left and a right udder quarter, taken at 24 h post-infection was serially diluted in PBST and tested with the *O. ostertagi* ELISA. The dilutions were 1/1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256 and 1/512.

2.6. Statistical analysis

The time evolution of the ODR for *O. ostertagi* and IgG was analysed by a mixed model with animal as random effect, challenge (yes/no) and time (12, 24, 72 and 144 h post-infection) as categorical fixed effects and baseline ODR at the time of infection as continuous covariate. The ODR of infected and uninfected quarters was compared at each time point using Bonferroni's multiple comparisons technique with a 5 % overall error rate. The mean difference between infected and uninfected quarters with the 95 % CI was recorded at each time point.

In the titration experiment, the 2 adjacent dilutions that had values below and above an ODR equal to 0.150 were interpolated to obtain the dilution factor at ODR= 0.150. The ratio of this factor between the infected and uninfected quarter was defined as the difference in dilution at an ODR equal to 0.150 between both quarters and was determined for each cow individually. The extent in which the bulk-tank milk ODR would be affected when milk from 1 or more infected quarters is added to the bulk tank was estimated by the formula $\frac{ODR \times (D-1) \times I}{2Q - I}$, where D is the mean difference in dilution of the tested animals at an ODR equal to 0.150 and Q is the total number of quarters from which I quarters are infected. This formula makes the assumptions that all cow quarters have the same ODR value when uninfected and that infected quarters have a drop in milk yield that is set to 50 % (Vangroenweghe *et al.*, 2005). A specific example is given for a herd size of 40 lactating cows.

3. Results

3.1. *O. ostertagi* ODR and IgG ODR

In total, 600 milk samples were analysed with the *O. ostertagi* and the IgG ELISA. The course of the *O. ostertagi* ODR over time is displayed in Fig. 3.1. The mean ODR value of the left udder quarters was significantly (*P*< 0.001) higher than of the right udder quarters at each sampling time post-infection. Overall, the mean difference in ODR values was 0.161 units (95 % CI: 0.129; 1.193) (*P*< 0.001). The largest difference was observed at 24 h post-infection with a mean difference of 0.251 units (95 % CI: 0.172; 0.330) (*P*< 0.001). The difference at 144 h after infection was 0.185 units (95 % CI: 0.106; 0.264) (*P*< 0.001).

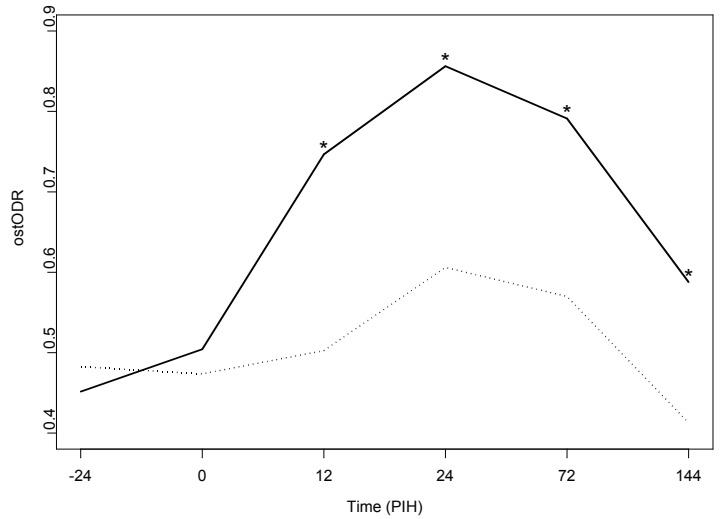

Fig. 3.1. Mean *O. ostertagi* ODR values of the milk samples at -24 h, 0 h, 12 h, 24 h, 72 h and 144 h post-infection [_____= left (infected) udder quarters; .....= right (uninfected) udder quarters; *= significant difference between left and right udder quarter (*P*< 0.001)].

The course of the IgG ODRs over the 6 sampling periods is shown in Fig. 3.2. An increase in the IgG ODR was observed at 24 h after infection with a mean difference between the left and the right udder quarters of 0.967 units (95 % CI: 0.873; 1.061) (*P*< 0.001). The ODRs decreased again at 72 h and 144 h after infection, but the differences between left and right udder quarters were still statistically significant (*P*< 0.001).

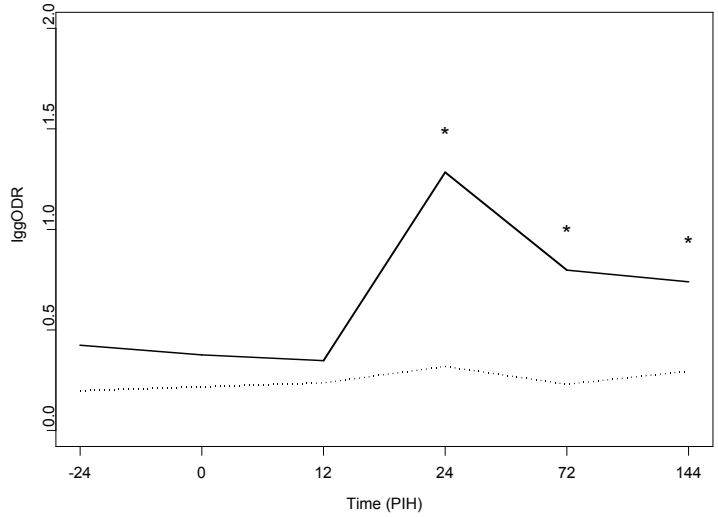

Fig. 3.2. Mean IgG ODR of the milk samples at -24 h, 0 h, 12 h, 24 h, 72 h and 144 h post-infection [_____= left (infected) udder quarters; .....= right (uninfected) udder quarters; *= significant difference between left and right udder quarter (P< 0.001)].

The Pearson correlation coefficients at 12 and 24 h post-infection of *O. ostertagia* ODRs and IgG ODRs with sodium and chlorine concentration and log transformed somatic-cell counts are shown in Table 3.1. All the correlations were highly significant except the correlation between *O. ostertagi* ODR and IgG ODR at 12 h post-infection (P= 0.06). The coefficients were low to moderate for both *O. ostertagi* ODR and IgG ODR at 12 h post-infection. At 24 h, the correlation coefficients increased for IgG ODR, but stayed moderate for the *O. ostertagi* ODR.

Table 3.1. Pearson correlation coefficients between *O. ostertagi* ODRs, total IgG ODRs, sodium concentration (mmol/l), chlorine concentration (mmol/l) and log transformed somatic-cell counts at 12 and 24 h post-infection.

	12 h post-infection				24 h post-infection			
	Ost. ODR	IgG	Na^+	Cl^-	Ost. ODR	IgG	Na^+	Cl^-
IgG	0.19[a]	-	-	-	0.31	-	-	-
Na^+	0.38	0.41	-	-	0.35	0.78	-	-
Cl^-	0.40	0.45	0.96	-	0.36	0.85	0.93	-
LogSCC	0.44	0.32	0.58	0.61	0.35	0.66	0.68	0.55

(all correlations significant at $P\leq$ 0.005, except for [a] where P= 0.06)

3.2. Titration experiment

A typical curve that was obtained after the titration of milk samples from a left and a right udder quarter taken at 24 h post-infection is shown in Fig. 3.3. The ODRs of the left udder quarters stayed at a plateau or increased to a maximum level during the first dilutions, whereas the ODRs of the right udder quarters decreased rapidly. The mean difference in dilution between left and right udder quarters at an ODR of 0.150 was 7.2 units (95 % CI: 4.2; 10.2) with a minimum of 1.9 and a maximum of 26.6.

The bulk-tank milk ODR of a herd consisting of 40 cows, with 159 healthy quarters and 1 infected quarter, was estimated to increase from 0.50 to 0.51 if the milk from this infected quarter was added to the bulk tank. Similarly, the bulk-tank milk ODR was estimated to increase from 0.50 to respectively 0.55 and 0.58 with 3 % and 5 % of the quarters infected.

Fig. 3.3. Titration curves of 2 milk samples taken at a left (infected) and a right (uninfected) udder quarter of an animal at 24 h post-infection.

4. Discussion

The present study demonstrates that an acute mastitis causes a flow of specific and non-specific antibody levels from serum to milk with a subsequent increase in the *O. ostertagi* ODR values of individual milk samples, which was largest at 24 h after experimental infection. The increase in ODR was an underestimation of the increase in specific antibody levels. This was demonstrated in the titration experiment, where a plateau or an apparent prozone-effect (Tizard, 2004) of the ODR values during the first dilutions was observed.

The *O. ostertagi* ODRs increased significantly starting at 12 h post-infection, whereas total IgG ODRs only increased from 24 h after infection. Moreover, the correlation coefficient between the 2 ODRs was low. This suggests that besides the flow of specific IgGs from serum to milk, there are other factors that play a role in the increase of the *O. ostertagi* ODR. A possible explanation is the cross-reaction of the *O. ostertagi* ELISA with other isotype antibodies.

The results demonstrate that milk from acutely infected quarters cannot be used for testing with the *O. ostertagi* ELISA until at least 6 days after infection. A rise, although smaller than in the infected quarters, was also observed in the *O. ostertagi* ODR values of the non-infected quarters (Fig. 3.1). This suggests that none of the quarters should be sampled in the presence of 1 infected quarter.

The effect of the contribution of milk from infected quarters on the bulk-tank milk *O. ostertagi* ODR was estimated to be minor if the relative number of infected quarters is small (< 3 %). However, the influence increased rapidly when the relative number of infected quarters was larger. This effect should be taken into account when a bulk-tank milk test result is used to evaluate the herd-infection level.

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CHAPTER 4

A survey to determine relationships between bulk-tank milk antibodies against *Ostertagia ostertagi* and milk-production parameters*

* Based on the manuscript: Charlier, J., Claerebout, E., Duchateau, L., Vercruysse, J., 2005. A survey to determine relationships between bulk tank milk antibodies against *Ostertagia ostertagi* and milk production parameters. Vet. Parasitol. 129, 67-75.

1. Introduction

In contrast to FSG calves, infections with GI nematodes in older cattle were for a long time considered to be of limited importance, merely due to the absence of clinical symptoms and the lower infection levels usually found in these animals. Several studies (Agneessens *et al.*, 2000; Borgsteede *et al.*, 2000) demonstrate however that GI nematodes are still widespread among adult cows in temperate-climate regions, with a prevalence of infection between 80-100 %. The most prevalent species was *Ostertagia ostertagi.* Moreover, 2 reviews suggest that subclinical GI-nematode infections in adult cows can have an effect on milk production. Gross *et al.* (1999) reported a significant increase in milk production after anthelmintic treatment in 70 of 87 experiments (80 %), with a median increase of 0.63 kg/cow per day. A meta-analysis of the milk production response after treatment in 75 experiments estimated an overall treatment effect of 0.35 kg/cow per day (Sanchez *et al.*, 2004b). Although there seems to be sufficient evidence to accept the impact of nematode infections on milk production, in many studies treatment responses show a large variation between different herds (Ploeger *et al.*, 1989, 1990; Walsh *et al.*, 1995; Kloosterman *et al.*, 1996). This might be due to differences in level of infection between herds (Ploeger *et al.*, 1989).

However, a major problem in adult animals is to determine a threshold infection level above which productivity is affected. Only antibody levels against *O. ostertagi* are considered as a promising parameter (Eysker and Ploeger, 2000). Previously, significant correlations were found between the mean of individual serum antibody levels and the bulk-tank milk antibody level (Kloosterman *et al.*, 1993; Sanchez *et al.*, 2002). Also, relationships were found between bulk-tank milk antibody levels and certain management practices known to be associated with infection levels, suggesting that bulk-tank milk antibody levels are a reasonable measure of parasite-infection levels in a dairy herd (Guitián *et al.*, 2000; Sanchez and Dohoo, 2002). In addition, a negative relationship was established between bulk-tank milk antibody levels and measures of productivity. However, previous work has been mainly done in Canada (Guitián *et al.*, 2000; Caldwell *et al.*, 2002; Nødvedt *et al.*, 2002; Sanchez

et al., 2002), where the herd-infection levels and the access to pasture appear to be lower than in West European studies (e.g. Agneessens *et al.*, 2000; Borgsteede *et al.*, 2000). Moreover, since milk production is influenced by many factors other than GI-nematode burdens, there is a need to confirm these previous results in large-scale studies. The objectives of the present study were (1) to investigate the relationships between bulk-tank milk anti-*O. ostertagi* antibody levels and milk production in a large-scale survey in Flanders, Belgium and (2) to investigate if seasonal variations in the antibody levels could affect this relationship.

2. Materials and methods

2.1. Selection of farms and sample collection

Out of a total population of 8,472 commercial Flemish dairy herds, 2,553 herds were randomly selected. From the selected herds, a bulk-tank milk sample was taken during the routine milk collection for the dairy cooperatives, in cooperation with the MCC Flanders. The herds were sampled on 2 occasions: once in April 2003 and once in September 2003. During the collection in April, all 2,553 herds were sampled, during the collection in September only 2,104 could be sampled. All samples arrived at the laboratory between 24 and 72 h after collection at the farms. During all handling procedures the samples were constantly cooled at 4 °C. After arrival at the laboratory, the milk samples were centrifuged (16,000 *g*, 5 min), fat was skimmed off and the supernatant was collected and frozen at –20 °C until further analysis.

2.2. Collection of farm and production data

Farm and production data were obtained from the milk-production recording programme of the Flemish Cattle Breeding Association (V.R.V., Vlaamse Rundveeteelt Vereniging).

The data were obtained from May 2002 to September 2003. The following herd-level variables were computed based on the individual test-day cow production data: monthly averages of kg milk/cow per day, milk-protein

%, milk-fat %, lactation number, DIM, somatic-cell count and the number of milk-producing cows, number of calvings and main breed. The main breed is defined as the breed that occurs most frequently, given that its proportion is higher than 80 %. Otherwise the main breed is mixed. The calving distribution over a year was computed out of the number of calvings. This variable was the ratio of the number of calvings in the months September 2002 to December 2002 and the number of calvings during the year before sample collection. The province of each herd was noted.

The number of months for which production data could be obtained differed between the herds. This was due to herds that stopped or began to participate in the milk-production recording programme during the investigated period. As a consequence, out of the 2,553 sampled herds in spring and the 2,104 sampled herds in autumn, the herds for which production data could be obtained differed for each investigated period (Table 4.1).

Table 4.1. Overview of sampling periods, number of sampled herds, periods over which relationships between milk production data and ODR were investigated and number of herds for which production data were available

Sampling period (number of sampled herds)	Investigated periods	Number of herds
Spring (2,553)	Year (May 2002 to April 2003)	1,063
	Summer (July 2002 to September 2002)	1,039
	Autumn (October 2002 to December 2002)	1,038
	Winter (January 2003 to March 2003)	1,039
	April (April 2003)	998
Autumn (2,104)	Year (October 2002 to September 2003)	867
	Autumn (October 2002 to December 2002)	848
	Winter (January 2003 to March 2003)	851
	Spring (April 2003 to June 2003)	851
	Summer (July 2003 to September 2003)	850
	September (September 2003)	756

2.3. Laboratory methods

The milk samples were thawed and recentrifuged (16,000 *g*, 5 min.) before analysis.

The *Ostertagia*-specific antibody levels were determined with an ELISA as described in chapter 2. The test results were expressed as an ODR. If a herd was sampled twice within a sampling period, the herd ODR was calculated by averaging the 2 ODR values.

2.4. Statistical analysis

The ODRs in spring (ODR_{spring}) and autumn (ODR_{autumn}) were compaired with each other by a 2-sided paired t-test using α= 0.05 as nominal significance level.

The effect of bulk-tank milk ODR on 3 different production parameters (milk production, milk-fat % and milk-protein %), was assessed by a multivariable linear regression. The multivariable model contained the covariates average lactation number, average DIM, main breed, average somatic-cell count, average number of producing animals and province because they are possible confounders.

The relationship between the ODR measured in April 2003 and September 2003 and the production parameters averaged over different periods was investigated. First, the association of ODR with the average production parameters (including kg milk-protein and kg milk-fat) in the month of sampling was studied with covariates also averaged out for that month. In this analysis also the calving distribution was included as a covariate. Second, the association of ODR with the average production parameters in the different preceding seasons was studied with covariates also averaged out for each particular season. The different seasons are defined in Table 4.1. Third, the effect of ODR on the average production parameters over the whole preceding year was studied with covariates also averaged out over a year.

Most herds had production data for each month. In the case of missing information for a particular month, the values were imputed by the mean value of the production parameter in that month over all farms multiplied with an adjustment factor. The adjustment factor corresponds to the ratio of the mean of the production parameter in the particular farm over the months for which information was available and the mean over those months over all other farms.

Finally, the effect of the change in ODR between April and September on the milk production was assessed by regressing the milk-production change on the ODR change.

3. Results

3.1. ODR values of the sampled herds

The average ODR_{spring} of the 2,553 sampled dairy herds was 0.825 with a range from –0.044 to 1.520 and a standard deviation of 0.201. The interquartile range of the ODRs (25^{th} to 75^{th} percentile) was 0.702 to 0.958. The average ODR of the 1,063 dairy herds for which production data were available was 0.806 with a range from –0.044 to 1.520 and a standard deviation of 0.202.

The average ODR_{autumn} of the 2,104 sampled dairy herds was 0.972 with a range from –0.133 to 1.899 and a standard deviation of 0.238. The interquartile range of the ODRs was 0.829 to 1.115. The average ODR of the 867 dairy herds for which production data were available was 0.942 with a standard deviation of 0.245 and a range from –0.133 to 1.899.

The ODR_{autumn} was significantly higher than the ODR_{spring} with a mean difference of 0.142 (P< 0.001). The ODRs of all the sampled dairy herds and the part of these herds for which production data were available are presented as box plots in Fig. 4.1.

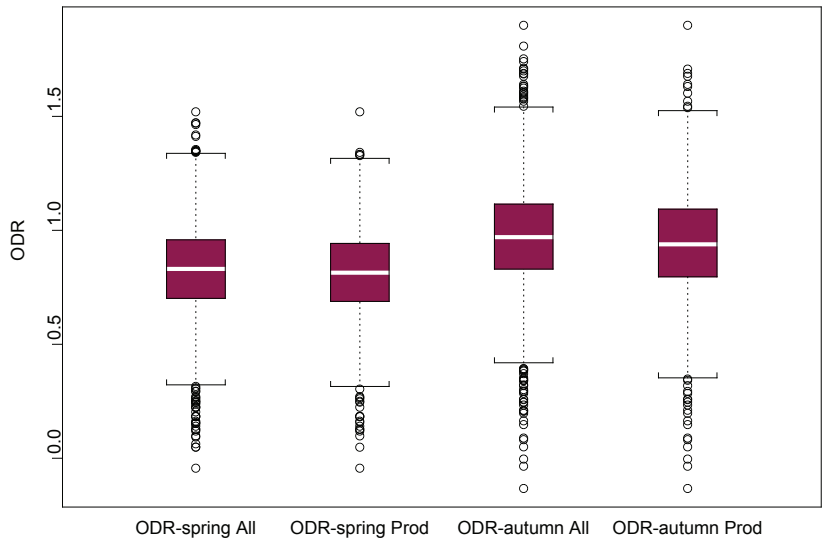

Fig. 4.1. Boxplots of the ODR values of all the sampled dairy herds in spring and in autumn (All), and the part of these herds for which production data were available (Prod).

3.2. Farm data

The herds for which production data could be obtained, were located in the 5 Flemish provinces. The herds were distributed approximately proportional to the total number of herds in each province. The main breed of the herds that were used in the analysis for ODR_{spring} over the period of a year, was classified as mixed for most of the herds (64.8 %), followed by Black Holstein (28.6 %), Red Holstein (4.8 %), Belgian White-and-red (1.3 %), Belgian Blue dual purpose (0.3 %), Belgian Red-pied (0.1 %) and Belgian Red (0.1 %). These proportions did not vary greatly in other investigated periods.

The average and standard deviation of the annual production parameters are displayed in Table 4.2.

Table 4.2. Mean and standard deviation (S.D.) of the milk-production parameters in the periods that were used to determine the relationship between ODR and milk yield, protein % and milk-fat % over the period of a year

Variable	May 2002 – April 2003		October 2002 – September 2003	
	Mean	S.D.	Mean	S.D.
Average milk yield (kg/cow per day)	22.9	4.84	23.3	4.80
Average milk-fat %	4.2	0.33	4.2	0.34
Average milk-protein %	3.4	0.15	3.4	0.15
Average lactation number	2.6	0.45	2.6	0.46
Average days in milk	192	37.9	194	38.2
Average number of producing animals	38	19.4	38	19.9
Average (somatic-cell count/1,000)	262	146.5	265	150.1

3.3. Relationship between ODR and milk yield

The regression coefficients for ODR of the multivariable analysis model for the different investigated periods are shown in Table 4.3. The regression coefficients of the possible confounding factors that were included in the model to determine the relationship between ODR_{spring} and annual milk yield are shown in Table 4.4. Significant effects were found for average number of producing animals, average DIM, average somatic-cell count, province and main breed. After controlling for these factors, significant negative linear relationships between ODR and milk yield were found for all the investigated periods. ODR_{spring} had a larger influence on average milk yield than ODR_{autumn}. For ODR_{spring}, the most negative regression coefficient was found in April and winter. For ODR_{autumn}, the most negative

regression coefficient was found in September and in summer. Over the period of a whole year an increase in the ODR_{spring} from 0.702 to 0.958 (interquartile range) was associated with a decrease in milk production of 1.1 kg/cow per day. Similarly an increase in ODR_{autumn} from 0.829 to 1.115 was associated with a decrease in milk production of 0.9 kg/cow per day.

When a herd's ODR increased between spring and autumn with 0.142 (average increase of all herds), it produced on average 0.4 kg/cow per day less in September than in April (slope= -2.93; $P< 0.001$), in comparison with herds where the ODR did not increase.

Table 4.3. Regression coefficients (and 95 % CI) of a multivariable linear-regression model to determine the relation of ODR with average milk yield (kg/cow per day) in different investigated periods

Model	ODR_{spring}	ODR_{autumn}
Autumn	-4.14 (-5.18; -3.11)	-3.14 (-4.07; -2.21)
Winter	-4.35 (-5.47; -3.22)	-3.04 (-4.06; -2.02)
Spring	/*	-2.97 (-3.84; -2.11)
Summer	-3.95 (-4.91; -2.99)	-3.56 (-4.45; -2.67)
April	-4.67 (-5.84; -3.50)	/**
September	/**	-3.58 (-4.60; -2.56)
Year	-4.28 (-5.24; -3.31)	-3.20 (-4.07; -2.32)

(all regression coefficients significant at level $P< 0.001$)

* No analysis was performed to determine the relationship between ODR_{spring} and the average milk yield in spring because the spring months before the sampling period (April 2003) are situated in 2 different calendar years.

** No analyses were performed to determine the relationship between ODR_{spring} and average milk yield in September and between ODR_{autumn} and average milk yield in April because this was irrelevant according to the study design.

Table 4.4. Regression coefficients, 95 % CI and *P*-values of the confounding factors included in the multivariable linear-regression model to determine the relation of ODR_{spring} with average annual milk yield (kg/cow per day)

Variable	Regression coeff.	95 % CI		*P*
		LL	UL	
Average number of producing animals	0.02	0.01	0.03	<0.001
Average lactation number	-0.36	-0.80	0.09	0.12
Average days in milk	-0.03	-0.04	-0.02	<0.001
Average somatic-cell count / 1,000	-0.01	-0.01	-0.01	<0.001
Province				
Limburg	Baseline			-
Antwerpen	-0.02	-0.65	0.69	0.95
Vlaams-Brabant	-0.59	-1.65	0.48	0.28
Oost-Vlaanderen	0.29	-0.35	0.92	0.38
West-Vlaanderen	2.19	1.46	2.92	<0.001
Main breed				
Red Holstein	Baseline			-
Mixed	-0.25	-0.96	0.46	0.49
Belgian Red-pied	-3.46	-7.84	0.94	0.12
Belgian Red	-7.60	-13.72	-1.48	0.02
Black Holstein	2.90	2.15	3.66	<0.001
Belgian White-and-red	-4.87	-6.51	-3.23	<0.001
Belgian blue dual purpose	-4.35	-8.03	-0.67	0.02

3.4. Relationship between ODR and milk-solids content

The regression coefficients of the multivariable analysis model for the different investigated periods are shown in Table 4.5 for milk-protein % and in Table 4.6 for milk-fat %. Significant negative linear relationships between ODR and milk-protein % were found in April and winter for ODR_{spring} and in summer, winter and spring for ODR_{autumn}. This resulted in a regression coefficient over the period of a whole year equal to –0.0273 (P= 0.06) for ODR_{spring} and to –0.0341 (P< 0.01) for ODR_{autumn}.

A significant positive association between ODR and average milk-fat % was found in summer for ODR_{spring} and in autumn for ODR_{autumn}. No significant associations were found in the other investigated periods, nor over the period of a whole year.

When ODR_{spring} was regressed against kg milk-protein and kg milk-fat in April, an increase in ODR_{spring} from 0.702 to 0.958 (interquartile range) was associated with a decrease of 0.044 kg protein/cow per day and 0.045 kg fat/cow per day. Similarly, when ODR_{autumn} was regressed against kg milk-protein and kg milk-fat in September, an increase in ODR_{autumn} from 0.829 to 1.115 was associated with a decrease of 0.037 kg protein/cow per day and 0.042 kg fat/cow per day.

Table 4.5. Regression coefficients (and 95 % CI) of a multivariable linear model to determine the relation of ODR with average milk-protein % in different investigated periods

Model	ODR_{spring}		ODR_{autumn}	
Autumn	-0.027	(-0.061; 0.007)	-0.011	(-0.042; 0.019)
Winter	-0.068[a]	(-0.106; -0.029)	-0.062 [a]	(-0.097; -0.028)
Spring	/*		-0.062 [a]	(-0.090; -0.034)
Summer	-0.020	(-0.054; 0.013)	-0.064 [a]	(-0.092; -0.035)
April	-0.049[c]	(-0.091; -0.006)	/**	/**
September	/**		-0.034	(-0.072; 0.003)
Year	-0.027	(-0.056; 0.013)	-0.034[b]	(-0.060; -0.008)

(a: $P< 0.001$; b: $P< 0.01$; c:$P< 0.05$)

* No analysis was performed to determine the relationship between ODR_{spring} and the average milk-protein% in spring because the spring months before the sampling period (April 2003) are situated in 2 different calendar years.

** No analyses were performed to determine the relationship between ODR_{spring} and average milk-protein % in September and between ODR_{autumn} and average milk-protein % in April because this was irrelevant according to the study design.

Table 4.6. Regression coefficients (and 95 % CI) of a multivariable linear model to determine the relation of ODR with average milk-fat % in different investigated periods

Model	ODR_{spring}		ODR_{autumn}	
Autumn	0.064	(-0.015; 0.143)	0.108[b]	(0.036; 0.180)
Winter	0.076	(-0.002; 0.154)	0.042	(-0.028; 0.112)
Spring	/*		0.005	(-0.064; 0.075)
Summer	0.086[c]	(0.005; 0.168)	-0.032	(-0.107; 0.044)
April	0.071	(-0.026; 0.168)	/**	
September	/**		-0.011	(-0.109; 0.086)
Year	0.049	(-0.018; 0.117)	0.037	(-0.027; 0.100)

(b: $P< 0.01$; c: $P< 0.05$)

* No analysis was performed to determine the relationship between ODR_{spring} and the average milk-fat % in spring because the spring months before the sampling period (April 2003) are situated in 2 different calendar years.

** No analyses were performed to determine the relationship between ODR_{spring} and average milk-fat % in September and between ODR_{autumn} and average milk-fat % in April because this was irrelevant according to the study design.

4. Discussion

As the sample size of herds was large and the herds were randomly selected over the different provinces, the study population can be assumed representative for the Flemish dairy population. Because of the similarity in the epidemiology of GI nematodes in the temperate-climate regions of Western Europe (Shaw *et al.*, 1998), it is likely that the results of our study can also be applied in these regions.

The mean ODR_{autumn} was significantly higher and had a larger spread than the mean ODR_{spring}. It is known that antibodies against *O. ostertagi* follow a seasonal pattern, with highest levels in late summer and autumn, and lowest in spring and early summer. This pattern follows the expected

epidemiological pattern of uptake of infective larvae (Agneessens *et al.*, 2000; Borgsteede *et al.*, 2000). Agneessens *et al.* (2000) described an increase in the mean serum ODR between April and September of 0.1. Sanchez and Dohoo (2002) observed an increase of the mean bulk-tank milk ODR between May and September of approximately 0.1. Our results are in agreement with these previous observations.

The relationship found between ODR and milk yield suggests that milk-yield losses due to GI nematodes in a dairy herd can be estimated by determination of bulk-tank milk antibody levels. The results over the period of a year are comparable with a previous report in Canada, where an increase in ODR from the 25^{th} to the 75^{th} percentile was associated with a drop in the milk production of 1.2 kg/cow per day (Sanchez and Dohoo, 2002).

The relationship with milk-production parameters was investigated for different periods. It seemed that the slope of the regression decreased with an increasing distance in time between the date of sample collection and the investigated period. This was clear for ODR_{spring}, and less clear for ODR_{autumn}. Given this fact, better associations were expected between ODR_{autumn} and milk yield in summer than between ODR_{spring} and milk yield in winter. During the summer the cows are exposed to larval challenge and the largest amount of adult *O. ostertagi* worms are present in the abomasum, while during the winter most of the worms are present as inhibited early L_4 (Agneessens *et al.*, 2000). However, the negative associations were stronger for ODR_{spring} than for ODR_{autumn}. A clear reason could not be found, although we can hypothesize that the dry weather conditions in the summer of 2003 have reduced the pasture infection levels and the influence of GI nematodes on milk production.

Although we controlled for several possible confounding factors in the analysis, the question remains whether there is a causal relationship between anti-*Ostertagia* antibody levels and milk production. Other effects like dilution of specific-antibody titers with increasing milk production (Kloosterman *et al.*, 1993) or mastitis could also cause a negative relationship between ODR and milk yield. Since transport of IgG to the mammary secretion is a receptor-dependent process (Butler, 1998), it is possible that this transport does not increase equally with milk production.

A dilution effect of milk yield on the IgG concentration in milk has been suggested by Watson *et al.* (1972) and Caffin *et al.* (1983). However, in a recent study it was suggested that individual ODR values are not greatly influenced by milk production (Sanchez *et al.*, 2004a). Also mastitis could bias the results. Serum total IgG concentrations are approximately 35-fold higher than total IgG concentrations in mature milk (Butler, 1986) and an infection of the udder can cause a flow of specific and non-specific antibodies from the serum to the milk (chapter 3; Guidry *et al.*, 1980; Caffin *et al.* 1983). However, acute mastitis cows generally do not contribrute to the bulk-tank milk and in this study we controlled for the subclinical mastitis effect by including the factor average somatic-cell count in the regression model.

A causal relationship between anti-*Ostertagia* antibody levels and milk yield can only be determined by a treatment trial that investigates whether there is a greater treatment response in high-ODR herds than in low-ODR herds. Sanchez *et al.* (2002) demonstrated an effect of the individual cow ODR on treatment response. High-ODR cows had an increase of 2.87 kg/day following treatment, while there was no apparent effect in low-ODR cows. However on the herd level no significant association between bulk tank antibody level and treatment response has thus far been demonstrated. A significant positive correlation between the mean herd milk-production response to treatment with the mean herd serum *Ostertagia* antibody titre was demonstrated by Ploeger *et al.* (1989). Kloosterman *et al.* (1996) found a larger treatment response in high bulk-tank milk antibody level herds than in low antibody level herds, although this difference lacked statistical significance.

The associations between ODR and milk solids are less uniform. Negative associations were found between ODR and milk-protein % during certain seasons and also over the period of a whole year. This suggests that GI-nematode infections also have a negative impact on the protein content of the milk. However, if we look at the size of the association, an increase in ODR_{autumn} over the interquartile range is associated with a decrease in milk protein concentration of only 0.01 %. No significant associations were found between ODR and milk-fat % over the period of a whole year. The positive associations that were found in some periods can be explained by

the dilution effect of milk yield on fat concentration of the milk. The associations with milk protein and fat concentration agree with 2 studies where no increase in these concentrations was found after anthelmintic treatment (Walsh *et al.*, 1995; McPherson *et al.*, 2001).

As a general conclusion, there exists in the Flemish dairy herds an important negative relationship between milk yield and GI-nematode infection level as estimated by the bulk-tank milk *O. ostertagi* ELISA, while there is no strong relationship between infection level and milk-solids content. More research should be done to investigate if the bulk-tank milk *O. ostertagi* ELISA can be used as a diagnostic tool to predict the milk-production response after anthelmintic treatment.

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CHAPTER 5

Associations between dairy herd management factors and bulk-tank milk antibody levels against *Ostertagia ostertagi*[*]

* Based on the manuscript: Charlier, J., Claerebout, E., De Mûelenaere, E., Vercruysse, J., 2005. Associations between dairy herd management factors and bulk tank milk antibody levels against *Ostertagia ostertagi.* Vet. Parasitol. 133, 91-100.

1. Introduction

The control of nematodes in cattle farms in temperate-climate regions relies almost solely on anthelmintic treatment. Modern anthelmintics are easy to administer, highly efficacious and the treatment schedules can be easily adapted to local management practices (Vercruysse and Dorny, 1999). Although anthelmintics will stay the cornerstone of nematode control in the coming decade(s), the non-chemotherapeutical control options will probably play an increasing role in the future. Recent reports indicate that cattle nematodes in the long term will develop resistance against MLs (Mejia *et al.*, 2003; Loveridge *et al.*, 2003). In addition, environmental issues (Lumaret and Errouissi, 2002) and the raising concern of the public opinion about food residues (O'Keeffe and Kennedy, 1998) are increasing the attention for control strategies that rely less on the use of anthelmintics.

Because of the absence of clinical symptoms due to GI nematodes, only a restricted or no use of anthelmintics is recommended in second grazing season animals and adult cows (Vercruysse and Claerebout, 2001). On the other hand, the economic costs of subclinical parasitism (Sanchez *et al.*, 2004a) and the continuing need to increase the productivity in modern dairy operations stimulate the farmers to apply parasite-control measures in adult dairy cows. Hence, there is a conflict between the striving for a reduced use of anthelmintics on the one hand, and the need for parasite control to increase productivity on the other hand. Besides the need for a diagnostic tool that can identify dairy herds that would benefit from nematode control, this evolution also necessitates the development of practical alternative control strategies for adult dairy cows. Different alternative control methods such as vaccines, biological control and genetically resistant hosts are currently being investigated but until now, only (grazing-)management practices can be used on the farms (Barger, 1996). However, in contrast to risk factors associated with GI-nematode infection level in first- and second-season grazing calves, which are well described (e.g. Hansen *et al.*, 1989; Shaw *et al.*, 1998a,b; Ploeger *et al.*, 2000), only little information is available on factors that have an impact on the infection level of adult dairy cows. Therefore, a questionnaire was

developed based on information available in the literature (Claerebout *et al.*, 2000; Leslie *et al.*, 2000; Guitián *et al.*, 2000; Sanchez and Dohoo, 2002; Caldwell *et al.*, 2002) to investigate the associations between herd-management factors and bulk-tank milk antibody levels against *Ostertagia ostertagi*, the most prevalent GI nematode in adult cows (Agneessens *et al.*, 2000; Borgsteede *et al.*, 2000). This is the first large-scale study that describes these associations under Western European conditions.

2. Materials and methods

2.1. Dairy farms and sample collection

This study was a cross-sectional survey. Out of a sampling frame of 8,472 commercial Flemish dairy herds, 2,104 herds were randomly chosen. From these herds only those that participated in the milk-production recording programme of the V.R.V. were selected for this study (n= 1,032).

In most of the Flemish dairy herds the cows have access to a pasture between April/May and October/November. The number of adult animals ranges from around 15 to 200, with a mean of 38 dairy cows. Most herds consist out of Holsteins or crossbreds with local breeds.

In September 2003, a bulk-tank milk sample was collected from the selected herds during the routine milk collection of the dairy cooperatives, in cooperation with the MCC Flanders. The samples were cooled at 4 °C until they arrived at the laboratory between 24 and 72 h after collection at the farm. After arrival at the laboratory, the milk samples were centrifuged (16,000 *g*, 5 min), fat was skimmed off and the supernatant was collected and frozen at –20 °C until further analysis.

2.2. Collection of herd information

A questionnaire was developed and tested in a pilot survey on 30 farms to evaluate the validity of the approach and the comprehensibility. Minor adjustments were carried out to achieve the final questionnaire. The questions were closed and concerned 3 subjects: general herd factors, pasture management and anthelmintic treatment. The description of all the questions is presented in Tables 5.1–5.3. The interviews were done in

December 2003 by the milk collectors of the V.R.V., who also filled in the questionnaire.

In addition, information on herd type was obtained from an external dataset (SANITEL). A herd was considered as "mixed" if at least 5 beef cows were present along with dairy cows, otherwise the herd was classified as "dairy". The herd mean somatic-cell count and the average lactation number of the cows in the month of sample collection were obtained from the milk-production recording database of the V.R.V.

Table 5.1. Description of the general herd factors and calf related factors with the proportion of herds and mean ODR per category

Variable	Description	*N*	Proportion (%)	Mean ODR
Herd type				
Mixed	Dairy and beef	262	34	0.975
Dairy	Dairy	507	66	0.931
Herd size	Number of adult dairy cows			
<30		189	24	0.969
30-60		414	53	0.961
>60		173	22	0.883
Calf_opinion	Opinion of the farmer on the			
Yes	presence of clinical symptoms in	74	10	0.964
No	the FSG calves due to GI	623	83	0.943
No opinion	nematodiasis	53	7	0.935
Calf_deworm	Anthelmintic treatment applied			
No	to the calves	85	13	0.925
Only if problems		89	13	0.947
Once a year		206	31	0.968
Twice a year		130	19	0.929
Bolus		164	24	0.954

2.3. ELISA procedure

The milk samples were thawed and recentrifuged (16,000 *g*, 5 min.) before analysis. The *Ostertagia*-specific antibody levels were measured with an ELISA as previously described in chapter 2. This ELISA uses a crude extract of adult *O. ostertagi* worms as antigen and the test results are expressed as ODRs.

2.4. Statistical analysis

Validation of the questionnaire was done by determining the Spearman rank correlation coefficient between the herd type as determined by the questionnaire and by the SANITEL-dataset.

Only the herds for which both an ODR value and a completed questionnaire were available were used in the following analyses. Two linear-regression models were used to evaluate the associations between herd-management factors (independent variables) and ODR values (dependent variable). The first model was based on the data from all the herds and was used to evaluate the effect of general herd factors and level of the cows' exposure to pasture on the ODR. The second model contained only the herds where the cows had access to a grassed area (paddock or pasture) and was used to evaluate the effect of pasture management factors and anthelmintic treatment related to different age categories (calves, heifers and cows). In this model, explanatory variables were screened by running a simple linear regression for each independent variable. A variable with $P< 0.05$ was considered to have passed the initial screening. Next, the Spearman rank correlation coefficients were computed between the variables that had passed the initial screening. When the correlation coefficient between 2 variables was higher than 0.32, a decision was made which of the 2 variables should be excluded from further analysis to avoid problems of multicollinearity. The final model was built up starting with all the variables that had passed the 2 screenings. Forward stepwise regression was used with a nominal significance level of $\alpha= 0.05$ and $\alpha= 0.10$ for the entry, respectively removal of a variable. The variables "cow_pasture" and "heifer_pasture" were recategorized with the categories "zero-grazing" and "yard" combined in 1 category due to a limited number of observations in the category "yard". Because there exists a positive correlation between somatic-cell count and ODR values (Sanchez *et al.*, 2004b; chapter 3) and to take into account that a higher level of immunity in older cows could reduce the impact of the management factors, the log transformed herd mean somatic-cell count and the average lactation number were included in both models as covariates.

Table 5.2. Description of the heifer related factors with the proportion of herds and mean ODR per category

Variable	Description	*N*	Proportion (%)	Mean ODR
Heifer_pasture				
Zero-grazing	Heifers were kept in a zero-grazing system	24	3	0.682
Yard	Heifers had access to a small area without grass	2	0	0.899
Paddock	Heifers had access to a small area with grass	59	8	0.984
Pasture	Heifers had access to pasture	661	89	0.954
Heifer_grazingtime	Grazing time per day of heifers			
24h		689	94	0.961
>6h		19	3	0.870
<6h		23	3	0.888
Heifer_turnout	Month of turnout on pasture of heifers			
March		21	3	1.013
April		430	59	0.983
May		250	35	0.919
June		22	3	0.855
Heifer_turnin	Month of turn-in on stable of heifers			
September		39	5	0.820
October		231	32	0.950
November		378	53	0.972
December		66	9	0.977
Heifer_pasturehistory	Animals that grazed on heifers' pasture during			
Calves	the previous year	2	0	0.835
Heifers		492	78	0.949
Cows		111	18	1.010
Other spp.		2	0	1.004
No animals		21	3	0.974
Heifer_rotation	Permanent pasture vs. rotational grazing			
Permanent		476	66	0.967
Rotational		247	34	0.934
Heifer_stocking rate	Stocking rate: number of heifers/ha			
<5		214	30	0.987
5-10		464	64	0.954
>10		44	6	0.845
Heifer_mowing	Mowing of heifers' pastures			
Yes, 50 to 100 %		226	31	0.909
Yes, <50 %		249	35	0.984
No		243	34	0.959
Heifer_manure	Spreading manure on heifers' pastures			
Yes, 50 to 100 %		222	31	0.913
Yes, <50 %		217	30	0.979
No		281	39	0.972
Heifer_deworm				
No	Heifers did not receive anthelmintic treatment	148	24	0.955
Spring	Group treatment in spring	205	33	0.961
Summer/Fall	Group treatment in summer or fall	264	43	0.959

Table 5.3. Description of cow related factors with the proportion of herds and mean ODR per category

Variable	Description	*N*	Proportion (%)	Mean ODR
Cow_pasture				
Zero-grazing	Cows were kept in zero-grazing systems	5	1	0.053
Yard	Cows had access to a small area without grass	6	1	0.749
Paddock	Cows had access to a small area with grass	164	21	0.941
Pasture	Cows had access to pasture	591	77	0.958
Cow_grazingtime	Grazing time per day of adult animals			
24h		467	61	0.989
>6h		221	29	0.897
<6h		72	9	0.878
Cow_turnout	Month of turnout on pasture of cows			
March		87	12	1.005
April		508	67	0.974
May		146	19	0.888
June		14	2	0.530
Cow_turnin	Month of turn-in on stable of cows			
September		42	6	0.833
October		259	34	0.933
November		407	54	0.970
December		45	6	1.008
Cow_pasturehistory	Animals that grazed on cows' pasture during the previous year			
Calves		2	0	0.883
Heifers		11	2	0.999
Cows		649	95	0.958
Other spp.		2	0	0.693
No animals		20	3	1.056
Cow_rotation	Permanent pasture vs. rotational grazing			
Permanent		296	39	0.960
Rotational		455	61	0.946
Cow_together	Grazing together of lactating cows with other age groups			
Yes, with calves		9	1	0.971
Yes, with heifers		35	5	0.906
No		711	94	0.954
Cow_stocking rate	Stocking rate: number of cows/ha			
<3		35	5	0.927
3-5		301	42	0.995
>5		377	53	0.919
Cow_mowing	Mowing of cows' pastures			
Yes, 50 to 100 %		335	44	0.896
Yes, <50 %		312	41	0.986
No		110	15	1.019
Cow_manure	Spreading manure on cows' pastures			
Yes, 50 to 100 %		286	38	0.925
Yes, <50 %		244	32	0.970
No		227	30	0.969
Cow_deworm				
No	Cows did not receive anthelmintic treatment	529	81	0.951
Spring	Group treatment in spring	25	4	1.012
Summer/Fall	Group treatment in summer or fall	64	10	0.917
Periparturient	Individual treatment around calving	33	5	0.945

3. Results

Information was obtained from 956 of the 1,032 selected herds (93 %). The mean response rate per question was 96 % with a range from 78 % to 100 %. The correlation coefficient between herd type as determined by the questionnaire and by the SANITEL-dataset was 0.73.

A bulk-tank milk sample could be obtained from 779 out of the 956 herds for which information was obtained. The mean ODR was 0.948 with a standard deviation of 0.239 and a range of –0.035 to 1.899. The mean ODR values for all the investigated categorical variables are shown in Tables 5.1 to 5.3.

The results of the model to evaluate general herd factors and level of exposure to pasture of the cows are presented in Table 5.4. Significant effects were found for all the factors in this model, except for the covariate average lactation number. The herd size was significantly associated with ODR values (P= 0.003). The ODRs in medium herds (30-60 cows) and small herds (< 30 cows) were on average respectively 0.075 (P= 0.001) and 0.058 (P= 0.03) units higher than in large herds. Herds with only dairy cows had lower ODRs (P= 0.02) as compared to herds where both dairy and beef cows were kept. Also the level of exposure to pasture of the dairy cows had a significant effect on the ODR (P< 0.001). Herds where the cows had no access to a grassed area had an ODR of on average 0.482 units lower (P< 0.001) than herds where the cows had access to a pasture. Herds where cows had only access to a grassed paddock had an ODR of on average 0.029 units lower than herds where the cows had access to a pasture, however this effect was not significant (P= 0.18).

The results of the model to evaluate pasture management practices are presented in Table 5.5. Later turnout on pasture of the cows and mowing the pastures of the cows were significantly associated with lower ODRs. When the cows had a progressively later turnout on the pasture, the ODR showed a gradual decrease. The average reduction in ODR associated with a turnout in June instead of March was estimated at 0.362 units (P< 0.001). Also a gradual effect of mowing the pastures of the cows was observed. Herds where most (all or > 50 %) of the pastures were mowed

had an ODR that was respectively 0.058 units (*P*= 0.002) and 0.081 units (*P*= 0.003) lower than herds where only a small part (< 50 %) or none of the pastures were mowed. The level of exposure to pasture of the heifers was significantly associated with the ODR. An increased level of exposure to pasture of the heifers was significantly associated with higher ODRs (*P*= 0.001). When the heifers had no access to a grassed area, the ODRs were on average 0.182 units (*P*< 0.001) lower than when the heifers had access to a pasture. There was no significant difference in ODR between herds where the heifers were kept on a paddock or on a pasture. A marginally significant effect was observed for grazing time per day of the cows. Cows that had a restricted grazing time per day tended to have lower ODR values than cows that grazed 24 h per day (*P*= 0.07).

Table 5.4. Results of the linear-regression model to evaluate the effect of general herd factors and of the level of the cows' exposure to pasture on bulk-tank milk antibody levels against *O. ostertagi* (R^2= 0.11; *n*= 669)

Variable	Regression coefficient	95 % CI	*P*-value
Log (mean somatic-cell count/1,000)	0.148	(0.059; 0.236)	0.001
Average lactation number	0.030	(-0.008; 0.068)	0.122
Herd size			0.003
>60	Baseline	-	-
30-60	0.075	(0.032; 0.119)	0.001
<30	0.058	(0.006; 0.111)	0.030
Herd type			
Mixed	Baseline	-	-
Dairy	-0.045	(-0.083; -0.008)	0.018
Cow_pasture			<0.001
Pasture	Baseline	-	-
Paddock	-0.029	(-0.072; 0.014)	0.183
No access to grassed area	-0.482	(-0.628; -0.337)	<0.001

Table 5.5. Results of the linear-regression model to evaluate the effect of pasture management practices and anthelmintic treatment on bulk-tank milk antibody levels against *O. ostertagi* (R^2= 0.17; *n*= 624)

Variable	Regression coefficient	95 % CI	*P*-value
Log (mean somatic-cell count/1,000)	0.126	(0.041; 0.211)	0.004
Average lactation number	0.028	(-0.008; 0.064)	0.133
Cow_turnout			<0.001
March	Baseline	-	
April	-0.008	(-0.061; 0.046)	0.781
May	-0.063	(-0.126; 0.001)	0.054
June	-0.362	(-0.496; -0.228)	<0.001
Cow_mowing			0.002
Yes, 50 to 100 %	Baseline	-	
Yes, <50 %	0.058	(0.020; 0.095)	0.002
No	0.081	(0.027; 0.135)	0.003
Heifer_pasture			0.001
Pasture	Baseline	-	
Paddock	0.032	(-0.027; 0.091)	0.292
No access to grassed area	-0.182	(-0.280; -0.0833)	<0.001
Herdsize			0.055
>60	Baseline	-	
30-60	0.046	(0.004; 0.089)	0.034
<30	0.010	(-0.042; 0.062)	0.719
Cow_grazingtime			0.068
24h	Baseline	-	
>6h	-0.044	(-0.084; -0.004)	0.030
<6h	-0.042	(-0.104; 0.021)	0.193

4. Discussion

This study describes the associations between herd-management factors and bulk-tank milk antibodies against *O. ostertagi*. Previously it has been demonstrated that there exists a negative relationship between bulk-tank milk antibody levels against *O. ostertagi* and annual average milk yield of a dairy herd (chapter 4). If it can be shown that this is a causal relationship, management factors that are associated with lower antibody levels can be implemented on farms to lower the GI infection levels and thus increase productivity.

In the first regression analysis (Table 5.4), a significant effect on ODR was found for the variables herd size and herd type. However, in the second regression model the effect of these variables was no longer significant,

suggesting that their effects are explained by the grazing management factors evaluated in this model. The level of exposure to pasture of cows was significantly associated with the ODR value. This agrees with all previous studies that were done in Canada (Guitián *et al.*, 2000; Sanchez and Dohoo, 2002; Caldwell *et al.*, 2002), indicating that it is one of the most important factors affecting the ODR. This observation fits in the trend of increasing intensification and dairy farms with permanent housing systems. However, in the highly specialised European dairy farms of today, outdoor grazing during summer is still the main practice (van Arendonk and Liinamo, 2003). Therefore, management changes that allow to some extent grazing of the cows will be of greater interest to most dairy farmers.

Time of turnout and mowing are well known risk factors for ostertagiosis in calves (Morley and Donald, 1980). For instance, it was demonstrated that postponing the turnout of FSG calves from mid-May to mid-June reduced the number of overwintered larvae that were picked up and prevented the development of parasitic gastroenteritis (Nansen *et al.*, 1987). The results of the present study indicate that postponing turnout and mowing can also be considered as important risk factors in cows. However, both variables were moderately related with respectively time of turnout of the heifers (R= 0.35) and mowing heifer's pasture (R= 0.44), which were not included in the model. Therefore we should be careful in assigning all of the observed effects to only the management of the cows.

The level of exposure to pasture of the heifers was significantly associated with the bulk-tank milk ODR. Since only lactating cows contribute to the bulk-tank milk, this seems a surprising observation. However, different heifer management factors were previously reported to be significantly associated with bulk-tank milk antibody levels (Guitián *et al.*, 2000; Sanchez and Dohoo, 2002; Caldwell *et al.*, 2002), suggesting that heifer management can affect the infection status of the adult cows. In the present study, heifers and adult cows were only grazing together in a small number of herds, making it unlikely that an increased infection level of the cows is caused by the higher egg output of the heifers on the cow's pasture. Moreover, in this study no effect on ODR was observed when the cows were grazing together with calves or heifers. This suggests that the

effect of the heifers' exposure to pasture on bulk-tank milk ODR is caused by the antibody response to the worm burden that is acquired before calving. In addition, L_3 ingested before calving can survive in hypobiosis (early L_4) and develop during the first lactation of the cow. In the Flemish dairy herds, primiparous cows constitute on average 25 % of the total herd, so it is likely that they can have an effect on the bulk-tank milk ODR.

Drenching programmes and pasture management of calves were previously reported to have an effect on the milk-yield gain of adult cows after anthelmintic treatment (Bisset *et al.*, 1987). However in the present study, no associations were found between the calf infection level as estimated by the farmer or anthelmintic strategy applied to calves and the specific antibody levels of the bulk-tank milk.

Also no associations were found between anthelmintic treatment of cows or heifers and ODR. Although it is known that serum antibody levels against *O. ostertagi* decrease after anthelmintic treatment (Berghen *et al.*, 1990), Sanchez *et al.* (2002) could not demonstrate an effect of treatment on milk antibody levels. Berghen *et al.* (1993) stated that antibody response is not a good criterion on which to base the impact of treatment, since the treatment effect can be masked by the stimulating effect of continuing intake of infective larvae.

The lack of association between ODR and time of turn-in confirms the observations of Shaw *et al.* (1998b) where no association was found between the length of the grazing season en the incidence of parasitic gastro-enteritis in calves. It has previously been reported that there is no consistent relationship between rotational grazing or stocking rate and parasite burden (Morley and Donald, 1980; Stromberg and Averbeck, 1999). This agrees with the results of the present study where no associations were found for both factors.

As a conclusion, the present study demonstrates that significant associations can be found between bulk-tank milk antibody levels against *O. ostertagi* and herd-management factors. The detected factors (restricted access to pasture, later turnout and mowing) should now be evaluated in field surveys to confirm if they are useful to control the parasite infection levels. Key points will be how easily these factors can be

incorporated in the general management, the costs related to changes in feeding and housing management and the effect on the selection of worms for anthelmintic resistance. As only a small part of the variation in ODRs could be explained by the analysed factors, it is likely that other management factors are of importance that were not investigated. Factors that could be of interest are additional feeding, production level of the cows and pasture species.

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CHAPTER 6

Predicting milk-production responses after an autumn treatment of pastured dairy herds with eprinomectin*

* Based on the manuscript: Charlier, J., Duchateau, L., Claerebout, E., Vercruysse, J., 2007. Predicting milk-production responses after an autumn treatment of pastured dairy herds with eprinomectin. Vet. Parasitol. 143, 322-328.

1. Introduction

Over the last decade, interest in deworming adult dairy cows has increased. This is merely due to the high prevalence of infection with GI nematodes in pastured dairy cows (Agneessens *et al.*, 2000; Borgsteede *et al.*, 2000), the marketing of anthelmintics with a zero-withdrawal time for milk (e.g. Shoop *et al.*, 1996) and the evidence that GI-nematode infections can have a negative impact on milk yield (Gross *et al.*, 1999; Sanchez *et al.*, 2004). However, routine treatment of all adult cows with anthelmintics cannot be recommended. First, in many studies treatment responses have shown a large variation between different herds, ranging from approximately -3 to 4 kg/cow per day (e.g. Ploeger *et al.*, 1989; Kloosterman *et al.*, 1996). Deworming will thus not be profitable in every herd. Second, routine application of anthelmintics to the adult cows could increase the selection pressure for anthelmintic resistance, as a larger part of the worm population will be exposed to anthelmintics (Coles, 2005). Consequently, there is a need for diagnostic measures that can identify the herds where the infection with GI nematodes negatively affects productivity (milk yield) and thus allow a targeted use of anthelmintics.

An ELISA that determines the antibody levels against *Ostertagia ostertagi* in milk has shown to be promising for this purpose. Sanchez *et al.* (2002; 2005) found evidence of a positive milk yield response to anthelmintic treatment in individual cows with a high antibody level, while there was no response in low antibody level cows. On the herd level, negative relationships have been established between the anti-*O. ostertagi* antibody level in bulk-tank milk and the annual average milk yield (Sanchez and Dohoo, 2002; chapter 4). However, a significant relationship between the bulk-tank milk antibody level and the production response after anthelmintic treatment has not been demonstrated yet. The objectives of this study were (1) to determine the effect of a whole-herd eprinomectin treatment in autumn on the anti-*O. ostertagi* bulk-tank milk antibody level; (2) to determine the overall effect of this treatment on milk-production parameters (milk yield, milk-protein % and milk-fat %) and (3) to investigate whether the pre-treatment *Ostertagia*-specific

bulk-tank milk antibody level was able to identify the herds with a positive milk-yield response.

2. Materials and methods

2.1. Herd Selection

One-hundred and nineteen herds were selected within the clientele of 7 veterinary practices in the provinces East- and West-Flanders, Belgium. The inclusion criteria for selection of the herds were: access to pasture of cows during summer months; participation in the milk production recording programme of the Flemish Cattle Breeding Association; no use of anthelmintics in the adult dairy cows during the last 6 months before the experimental treatment and no evidence of infections with ectoparasites in the adult cows.

2.2. Treatment Protocol

The herds were rank ordered from lowest to highest based on their *Ostertagia*-specific bulk-milk antibody level in August 2004 as the first blocking factor and on their rolling herd average milk production in the month of July 2004 as the second blocking factor. Within blocks of 2, the herds were randomly assigned to either the treatment or the control group. The treatment group received a topical administration of eprinomectin (Eprinex Pour-On, Merial) at a dosage of 500 μg/kg (1ml/10 kg) and the control group received a placebo consisting of the vehicle liquid without the active compound (1ml/10 kg). Treatments were done double blind, i.e. neither the veterinarian applying the treatments, nor the farmer knew if a herd was treated with eprinomectin or the placebo. This was guaranteed by dispensing the eprinomectin and placebo in identical bottles that were uniquely labelled with a letter. The treatment was administered in the month of October 2004 to all the adult cows (lactating and dry) and the heifers that would calve within the first 5 months after treatment.

2.3. Sample collection and ELISA-procedure

Bulk-tank milk samples were collected in cooperation with the MCC Flanders. The samples were collected monthly during the routine milk collection by the dairy cooperatives from August 2004 to April 2005. All samples were kept at 4 °C until arrival at the laboratory between 24 and 72 h after collection at the farms. Next, the milk samples were centrifuged (16,000 *g*, 5 min), fat was skimmed off and the supernatant was collected and frozen at -20 °C until further analysis.

The samples were analysed with an *O. ostertagi* milk ELISA using a crude adult-worm antigen, as described in chapter 2. The test results were expressed as ODRs.

2.4. Collection of farm data

Milk-production data were obtained from the milk-production recording programme of the Flemish Cattle Breeding Association. The following monthly herd-level data were computed or registered based on the individual test-day records: kg milk/cow per day, milk-protein %, milk-fat %, lactation number, days in milk, somatic-cell count, number of milk-producing cows, number of calvings, main breed and province. The main breed is defined as the breed of at least 80 % of the stock, otherwise the main breed is classified as mixed. The calving distribution was computed as the ratio of the number of calvings in the months September 2004 to December 2004 and the number of calvings during the year-period May 2004 to April 2005.

At the beginning of the study, a form was distributed to the farmers to register additional antiparasitic treatments during 6 months after the experimental treatment. In the cases this form could not be recollected, the farmers were contacted by phone. In April 2005, a questionnaire was distributed to collect information on a series of parameters concerning feeding management (feeding method, concentrate supplier, feeding level in relation with milk quota) and health status (disease outbreaks and average percentages of abortions, cows with increased somatic-cell count, retained afterbirths and lame cows) in the year before and 6 months after the anthelmintic treatment.

2.5. Statistical analysis

The effect of treatment on ODR was analysed by a mixed model with herd as a random effect and treatment (yes/no), month and an interaction term between treatment and month as fixed effects.

The overall treatment effect on 3 production parameters (milk yield, milk-protein %, milk-fat %) was estimated by a mixed model using the monthly production of the 4 months after treatment. The analysis was restricted to 4 months after treatment to limit the effect of herds where milk yield was lowered in order to not exceed the milk quota. The variables month, treatment (yes/no) and the mean production in the 4 months preceding the treatment were introduced as fixed effects and herd as a random effect. Two-way interactions between the treatment effect with the average lactation number, average days in milk and the rolling herd average milk production were tested in separate models.

The effect of the pre-treatment bulk-tank milk ODR (of samples taken in September 2004) on the milk-production response after anthelmintic treatment was investigated by subdividing the farms in 6 categories based on their pre-treatment ODR values (category 1: 0-10 % of the data; category 2: 10-25 %; category 3: 25-50 %; category 4: 50-75 %; category 5: 75-90 % and category 6: 90-100 %). The treatment effect within each group was estimated by running the previously specified model, used to estimate the overall treatment effect, separately for each group.

The questionnaire data were evaluated by 2-sided Chi-squared tests, or when the expected counts in a cell were less than 5 by 2-sided Fisher's exact tests (significance level 0.05) to detect significant differences in feeding strategy and disease parameters between the eprinomectin and placebo group in the period after treatment. The same statistical tests were applied to check if changes between the period before and after treatment occurred more in 1 of the 2 groups (eprinomectin vs. placebo).

3. Results

3.1. Farms and animals

An anthelmintic treatment was applied to the dairy cows during the period of follow-up on 4 herds. After exclusion of these herds for all the statistical analyses, the eprinomectin and placebo group consisted of 59 and 56 herds, respectively. Additionally, there were 5 herds for which no production data could be retrieved. As a consequence, in the analyses to assess the overall treatment effect on milk production and the predictive value of the bulk-tank milk ODR, the eprinomectin and placebo group consisted of 57 and 53 herds respectively with 2,292 and 2,034 lactating animals present in October 2004.

The distribution of eprinomectin- and placebo-treated herds per province and main breed is given in Table 6.1. Average lactation parameters in the 4 months before and 4 months after treatment administration are given in Table 6.2. The mean calving distribution ± standard deviation was 0.46 ± 0.17 in the placebo group and 0.48 ± 0.17 in the eprinomectin group. The smallest and largest herd in the study counted on average 16 and 87 lactating cows respectively in the 4 months after treatment.

Questionnaires were obtained from 110 of 119 selected herds (92 %). No significant association could be detected for any of the questionnaire variables with treatment group (eprinomectin vs. placebo) in the period after treatment administration. Also, changes in the questionnaire variables between the period before and the period after treatment administration did not occur significantly more in one of the treatment groups.

Table 6.1. Location and main breed of the herds that were used in the analyses to evaluate the effect of eprinomectin treatment on milk yield (*n*= 110)

Variable	Eprinomectin	Placebo
Province		
East-Flanders	34	38
West-Flanders	23	15
Main breed		
Mixed	19	17
Black Holstein	32	31
Red Holstein	6	4
Belgian White-and-Red	0	1

Table 6.2. Herd averages (and standard deviation) of lactation parameters in the 4 months before and after the experimental treatment administration (*n*= 110)

Variable	Before treatment		After treatment	
	Eprinomectin	Placebo	Eprinomectin	Placebo
Milk yield (kg/cow per day)	24.4 (4.3)	24.1 (3.8)	27.8 (5.0)	26.3 (4.5)
Milk-protein %	3.36 (0.12)	3.34 (0.11)	3.39 (0.12)	3.36 (0.13)
Milk-fat %	4.12 (0.28)	4.08 (0.30)	4.28 (0.28)	4.27 (0.30)
Herd size	38 (12)	41 (15)	41 (14)	43 (16)
Lactation number	2.6 (0.3)	2.6 (0.4)	2.6 (0.3)	2.6 (0.4)
Days in milk	206 (36)	206 (32)	176 (28)	182 (31)
Somatic-cell count/1,000	275 (128)	267 (132)	218 (113)	244 (130)

3.2. Anti-*Ostertagia* antibody levels

Before the experimental treatment, there was a considerable between-herd variation in the anti-*Ostertagia* bulk-tank milk antibody levels. In the month September, the mean ± standard deviation (minimum; maximum) antibody level was 0.70 ± 0.13 (0.25; 0.97) ODR in the placebo group and 0.68 ± 0.15 (0.14; 1.06) ODR in the eprinomectin group. The course of the antibody levels across the study period is shown in Figure 6.1. There was a significant effect of the treatment ($P< 0.001$), with the ODR values being lower in the eprinomectin group at each time point after treatment. There was also a month effect ($P< 0.001$) and an interaction between month and treatment ($P< 0.001$): the antibody levels decreased in both groups from August until January with a greater decrease in the eprinomectin group and started to increase again in February/March. The largest differences between the 2 groups were observed in the months February and March with a difference of 0.15 ODR.

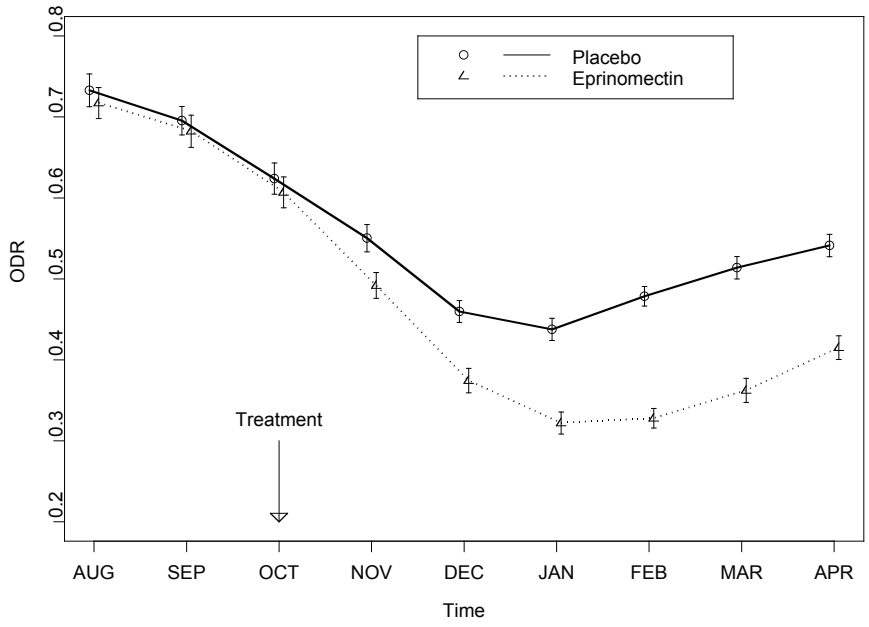

Fig. 6.1. The course of the *Ostertagia*-specific antibody levels in bulk-tank milk samples from 2 months before until 6 months after the experimental treatment in the eprinomectin and placebo group. Bars represent the standard error of the mean.

3.3. Overall treatment effect on milk yield

The overall treatment effect results on milk yield over the 4 months after treatment are shown in Table 6.3. There was a significant effect of the month ($P< 0.001$) and the average milk yield in the 4 months before treatment ($P< 0.001$) and a marginally significant effect of treatment ($P= 0.07$), with an average production of 27.6 kg/cow per day in the eprinomectin group and 26.4 kg/cow per day in the placebo group. The difference in milk yield between both groups was consistent over the 4 months following treatment, ranging from 0.9 kg/cow per day in November to 1.4 kg/cow per day in February. There were no significant interactions of the treatment effect on milk yield with the average days in milk ($P= 0.55$), the average lactation number ($P= 0.72$) and the production level of the herds ($P= 0.68$).

There was no treatment effect on the solids content of the milk. The estimates for the effect of treatment on the average milk-protein % and milk-fat % were respectively 0.029 % (95% CI: -0.009; 0.066) ($P= 0.13$) and 0.001 % (95 % CI: -0.092; 0.095) ($P= 0.98$).

Table 6.3. Regression coefficients, 95 % CI and *P*-values from a linear mixed model to determine the effect of a whole-herd treatment in autumn with eprinomectin on the milk yield (kg/cow per day) over the first 4 months after treatment

Variable	Regression coefficient	95 % CI		*P*-value
		LL	UL	
Intercept	6.27	1.87	10.66	0.006
Milk yield before treatment	0.81	0.64	0.99	<0.001
Treatment				0.070
No	Baseline	-	-	
Yes	1.21	-0.09	2.50	
Test month				<0.001
November	-1.14	-1.64	-0.64	
December	Baseline	-	-	
January	0.94	0.45	1.43	
February	1.06	0.57	1.55	

3.4. The relation between the pre-treatment anti-*O. ostertagi* antibody level in the bulk-tank milk and the treatment effect

The boundaries for the different ODR categories were for category 1: < 0.50 ODR, category 2: 0.50-0.63 ODR, category 3: 0.63–0.69 ODR, category 4: 0.69-0.77 ODR, category 5: 0.77-0.84 ODR and category 6: > 0.84 ODR. The treatment effect on milk yield within each category is given in Figure 6.2. The largest treatment effect was found in the herds with the highest pre-treatment ODR (> 0.84), and equaled 4.0 kg/cow per day (95 % CI: [1.0; 7.0], *P*= 0.03). The 95% CI of the other categories all included 0.

4. Discussion

In the present trial, whole-herd treatments were applied in the month of October. This approach was chosen (1) because of the ease of application (all animals at once), (2) because a treatment in autumn is expected to result in a maximum period with limited levels of infection as there will be no re-infection during the stabling period and (3) due to a slight-seasonal calving pattern in the Flemish dairy herds, this is the period when most cows are in early lactation. Ploeger *et al.* (1989) have suggested that greater economic benefit may be achieved when cows are treated at calving or early in lactation when the physiologic demands and energy requirements of the cows are highest. On the other hand, Block and Gadbois (1986) stated the importance of treating all cows at once to limit the level of reinfections.

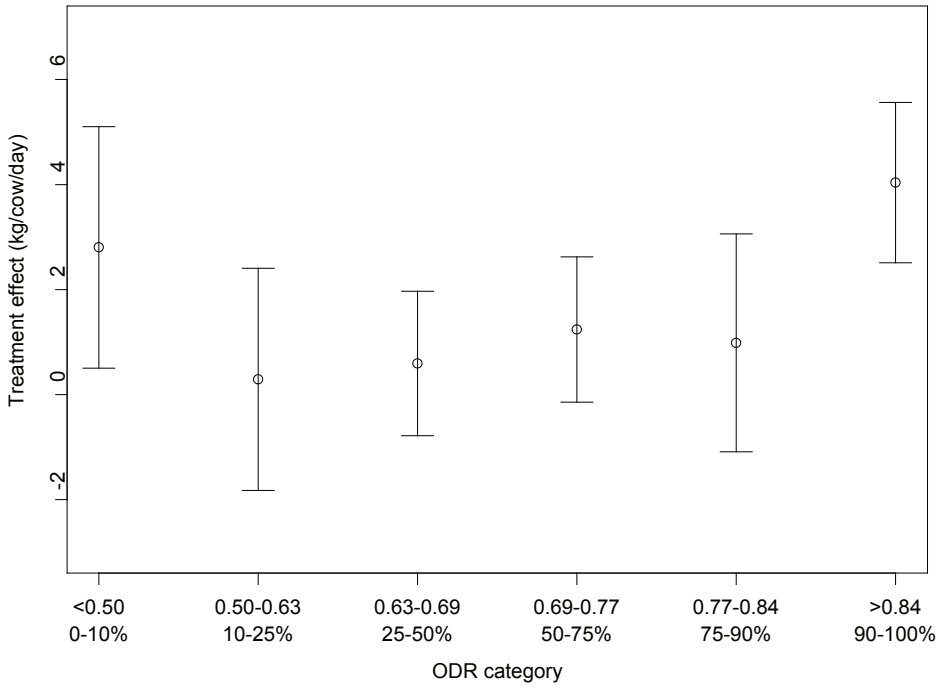

Fig. 6.2. The effect on milk yield (treatment effect) of a whole-herd eprinomectin treatment on 110 dairy herds in autumn within 6 categories of *Ostertagia*-specific bulk-tank milk antibody levels in the month before treatment. Percentages indicate the percentiles of the ODR values. Bars represent the standard error of the mean.

The between-herd variation that was observed in the pre-treatment anti-*Ostertagia* antibody levels was comparable with the variation observed in a previous survey (chapter 4). Both in the placebo and eprinomectin treated herds, the *Ostertagia*-specific antibody level decreased between September and January, with a greater decrease in the eprinomectin group. From February onwards, a steady increase in the ODR values was observed in both groups. In the placebo group, this can be explained by the antibody response evoked by inhibited-L_4 of *O. ostertagi* resuming their development (Claerebout *et al.*, 1997). The increase of the ODR values in the eprinomectin group is rather surprising, but can be explained by the fact that many herds still had limited access to pasture after the end of the duration of the persistent activity of eprinomectin against GI nematodes, allowing some re-infection.

The overall effect on milk yield was estimated at 1.2 kg/cow per day and this effect was consistent over the first 4 months following treatment. Previous studies have estimated the effect of eprinomectin treatment on

milk yield in dairy herds as 0.4, 0.9 and 2.4 kg/cow per day and this effect was in each study consistent over the study period (McPherson *et al.*, 2001; Nødvedt *et al.*, 2002; Reist *et al.*, 2002). Differences in study design, the time span over which the effect is measured and geographical differences in levels of infection and prevalence of parasitic species may account for the variation between the trials. Consistent with the previous trials no effect could be observed of treatment on the protein or fat content of the milk.

This study demonstrates that bulk-tank milk antibody levels against *O. ostertagi* have some value to identify herds that will have a positive milk-production response after anthelmintic treatment. Previously, Ploeger *et al.* (1989), found a significant positive correlation between the mean herd *Ostertagia* serum antibody titre and the herd's milk-production response to anthelmintic treatment. All studies investigating the value of bulk-milk antibody titres found higher responses to anthelmintic treatment in high antibody level herds than in low antibody level herds, but the differences lacked statistical significance (Kloosterman *et al.*, 1996; Sithole *et al.*, 2005). This may be due to the small sample size in the study of Kloosterman *et al.* (1996) and the generally low levels of infection in the study of Sithole *et al.* (2005), where the studied herds had no or a limited outdoor exposure.

The greatest treatment effect on milk yield was, as expected, observed in the herds with the highest pre-treatment anti-*Ostertagia* ODR. However, the next greatest effect was in the herds with the lowest pre-treatment ODR. Although we don't know the reason, this effect could be due to the removal of subclinical ectoparasite infections (e.g. *Chorioptes bovis*) by the eprinomectin treatment. While there was no evidence of ectoparasite infections in the herds, subclinical infections might still have been present. In addition, herds with a limited outdoor exposure have mostly very low ODR values (chapter 5) and are most susceptible to *Chorioptes* infections (Beck *et al.*, 2005). Smith (1997) stated that the relationship between parasitism and production loss in ruminants is very non-linear due to the multicausal nature of production losses and that the correlations between indices of parasitism and production losses may exist only at particular times in the infection cycle. Therefore, the results of this survey must be

interpreted according to the study design. We can only claim a predictive value on the milk-yield response after anthelmintic treatment of the bulk-tank milk ODR when samples are collected in the period September/beginning of October and when a whole-herd anthelmintic treatment is applied. If samples are taken later, the proposed cut-off (0.84 ODR) might not be applicable due to the seasonal pattern of the antibody levels. In addition, it is recommended to confirm the observed results in another study and to investigate the predictive value of anti-*Ostertagia* antibody levels in different management and climatic conditions. Previously, significant associations have been established between management factors and *Ostertagia*-specific bulk-tank milk antibody levels (Sanchez and Dohoo, 2002; chapter 5). The investigation of possible interactions between management factors and the predictive value of anti-*Ostertagia* antibody levels could lead to a better interpretation of the test results.

5. Conclusions

This randomised study demonstrates that a treatment with eprinomectin of cows around housing in pasture-based dairy systems will lower the *Ostertagia*-specific bulk-tank milk antibody level during the stabling period and can result in a consistent increase in milk yield over the following months. The effect of eprinomectin treatment on milk yield, however, differed as a function of the pre-treatment anti-*O. ostertagi* antibody level, with the largest milk yield increase observed in the herds with the highest antibody level. The results indicate that an *O. ostertagi* bulk-tank milk ELISA can be used to identify the herds where the greatest milk yield response after anthelmintic treatment is expected and can contribute to a strategic and justified use of anthelmintics.

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CHAPTER 7

General discussion: the use of milk antibodies to evaluate parasite-associated production losses in dairy cattle

1. Introduction

The general objective of this thesis was to use *Ostertagia ostertagi*-specific antibody levels in bulk-tank milk to study the epidemiology and impact on production of GI-nematode infections in dairy herds.

The performed studies showed that (1) the indirect crude antigen *O. Ostertagi* ELISA is a repeatable and robust technique; (2) there exist negative associations between the *Ostertagia* ODR in bulk-tank milk and milk-production parameters and (3) certain management practices are associated with the *Ostertagia* ODR. Moreover, there was evidence that the *Ostertagia* ELISA on bulk-tank milk can to some extent identify the herds where a positive production response after anthelmintic treatment is expected. Consequently, the obtained results indicate that *Ostertagia*-specific antibody quantification in bulk-tank milk can be highly relevant to monitor the GI-nematode infection level of the adult herd. However, additional studies are required to confirm the predictive value on the milk-production response after anthelmintic treatment.

In the next paragraphs, we will discuss possible uses of the *Ostertagia* ELISA by veterinarians and by researchers. More specifically, the objectives of this chapter are to discuss (1) the practical use by veterinarians of the *Ostertagia* ELISA on bulk-tank milk; (2) the limitations/advantages of testing bulk-tank milk versus individual milk; (3) the integration of monitoring GI-nematode infections in a broader dairy health context and (4) the use of milk ELISAs and geographical information systems (GIS) to determine the regional economic importance of parasitic diseases.

2. The practical use by veterinarians of the *Ostertagia* ELISA on bulk-tank milk

In this thesis both retrospective studies (chapter 4 and 5) and a prospective study (chapter 6) were performed. In the retrospective studies, highly significant associations were observed of the *Ostertagia* ODR on bulk-tank milk with annual average milk yield and management practices. In the prospective study however, the relation between the pre-treatment *Ostertagia* ODR and the milk-yield response after anthelmintic

treatment was less clear. The results from both types of study are not contradictory. However, their comparison points out the difference between associations of *Ostertagia* ODR with production parameters observed in a large population versus predictive associations on the individual farm level.

Despite this fact, the results of the retrospective studies indicate that the *Ostertagia* ODR, determined at the end of the grazing season can be used to evaluate the applied parasite control measures (e.g. pasture management, treatment programme) during the finished grazing period. High ODR values point out that the exposure to GI nematodes during the grazing season was high and that milk-yield losses have likely occurred. The prospective study indicates that the *Ostertagia* ODR determined at the end of the grazing period can be incorporated in the decision-making process whether an anthelmintic treatment around stabling is likely to result in a production gain. The definition of a clear threshold "to treat or not to treat" however, is less evident. For the end user, veterinarian or farmer, this may be considered as a drawback. On the other hand, thresholds for predicting the production gain after anthelmintic treatment should only be regarded as indicative for several reasons. (1) Production traits are highly variable and affected by many known and unknown factors. An event or interplay of several factors with a large impact on performance may disrupt the relationship between parasitism and production (Smith, 1997). (2) The effect of climate and geography on the parasite's epidemiology may interact with the relationship between parasite-specific antibody levels and productivity. Ploeger *et al.* (1990) hypothesized that a delayed uptake of infective larvae due to climatic conditions precluded the positive relationship between *Ostertagia*-antibody titre and milk-yield response to anthelmintic treatment that they observed a year earlier. (3) Genetic variation between animals can cause a different level of antibody response on the same larval challenge (Gasbarre *et al.*, 1993). On the herd level these variations will be less important, although differences between breeds may exist.

Another drawback may be the cross-reactivity of the crude *Ostertagia* antigen with other parasites. Cross-reactivity of the *Ostertagia* antigen with *Fasciola hepatica* and *Dictyocaulus viviparus* has been suggested, but

the extent to which this occurs and its relevancy for the interpretation of the test results have not yet been precisely defined (Ploeger *et al.*, 1994). However, it may not be worthwile to develop a more specific ELISA because the use of highly specific antigens seems to decrease the correlation with the larval exposure (e.g. de Graaf *et al.*, 1994). In addition, specific ELISAs for the detection of milk antibodies against *F. hepatica* (Reichel *et al.*, 2005; Salimi-Bejestani *et al.*, 2005) and *D. viviparus* (T. Schnieder, personal communication) are now available. In case of doubt, simultaneous testing of a bulk-tank milk sample against *O. ostertagi*, *F. hepatica* and *D. viviparus* could be performed to identify such cross-reactivity.

A proper way to assist the veterinarian in the interpretation of the *Ostertagia* ELISA results could be to impose the uncertainty on a positive milk-production response after anthelmintic treatment directly on the test result. A similar approach has been successfully applied for other infective agents by relating OD with probability of infection (Irion *et al.*, 2002; Toft *et al.*, 2005). This would mean that instead of working with a specific threshold, the probability of a production gain after treatment is given for a specific ODR. In combination with the herd's anamnesis and performance targets the veterinarian could then decide whether anthelmintic treatment is useful or not. However, more analyses and studies are needed to achieve this.

Other studies that could lead to a better interpretation of the test results are those looking into (1) possible interactions between *Ostertagia* ODR and other diseases or management factors on productivity; (2) the effect of climatic and geographical factors on the *Ostertagia* ODR and its relationship with productivity and (3) breed differences in the *Ostertagia*-specific antibody response.

3. The limitations/advantages of bulk-tank milk versus individual milk

In this thesis, the focus was on the use of bulk-tank milk. The major advantage of using bulk-tank milk is that it is a consumer-friendly and low-cost method. The samples can be easily obtained by the veterinarian

or by cooperation with milk-quality control centres. These facts, in addition with the results from previous studies (e.g. Sanchez and Dohoo, 2002) and this thesis indicate that the *O. ostertagi* ELISA on bulk-tank milk is a suitable method to evaluate the average exposure of the herd to GI nematodes. A limitation of using bulk-tank milk is that information is only obtained from the cows that are contributing to the bulk tank, ignoring cows in the dry period. Therefore, the test results of bulk-tank milk samples taken only a few weeks apart can show considerable variations depending on calving pattern, the number of cows contributing to the tank and their relative milk yields and antibody titres (Pritchard, 1998). Consequently, ELISA results based on bulk-tank milk should always be regarded as a rather "crude" parameter. Examples are indirect *F. hepatica* ELISAs on bulk-tank milk that only tested positive if at least 25 to 60 % of the dairy cows were infected (Reichel *et al.*, 2005; Salimi-Bejestani *et al.*, 2005).

Analysis of individual milk samples has the major advantage that they not only reflect the average herd exposure (e.g. by taking the average of the individual ODRs), but also the within-herd variability. It has been demonstrated that within herds with both a low or high bulk-tank milk ODR, there is a large variation of individual ODRs (Sanchez *et al.*, 2002; Charlier *et al.*, 2007a). Moreover, Sanchez *et al.* (2002; 2005) have demonstrated that the individual ODR determined in late lactation has a predictive value on the milk-production response after anthelmintic treatment at calving. Therefore, individual ODR determination could be used to develop selective anthelmintic treatment programmes, targeting only the high-ODR animals within a herd. Selective treatment programmes are currently proposed to slow down the development of anthelmintic resistance (Jackson and Miller, 2006). A disadvantage of individual milk samples is that the individual milk *Ostertagia* ODR is influenced by non-parasitic factors that hamper the interpretation. These factors are milk yield, season, mastitis, parity, stage of lactation and genetics. According to Kloosterman *et al.* (1993) and Sanchez *et al.* (2004), the factors with the largest effect are milk yield and age. A way to remove these effects on the individual ODRs would be to calculate adjusted ODRs based on correction coefficients (Sanchez *et al.*, 2004).

Finally, a possibility would be to combine *Ostertagia* ODR determination in individual and bulk-tank milk. First, high-ODR farms could be identified by bulk-tank milk analysis. Next, high-ODR farms could be advised to perform individual testing of the animals to allow targeted anthelmintic treatments. This would prevent the costs of testing all the animals on low-ODR farms. However, a cost:benefit analysis that takes into account the missed production gains in the low-ODR farms and analytic costs in high-ODR farms should determine if such an approach is valid.

4. The integration of monitoring gastrointestinal-nematode infections in a broader dairy-health context

Until now, most studies have considered GI-nematode infections as an isolated disease entity. However, it is generally accepted that almost every disease in cattle has a multifactorial nature. Therefore, a next step would be to integrate monitoring of GI-nematode infections in a broader dairy health context.

Studies that quantify production losses associated with subclinical infections with BVD virus (Fourichon *et al.*, 2005), bovine leukaemia (BL) virus (Ott *et al.*, 2003), *Mycobacterium avium* subspecies *paratuberculosis* (MAP) (Nordlund *et al.*, 1996), *Neospora caninum* (Bartels *et al.*, 2006b) and *F. hepatica* (Charlier *et al.*, 2007b) are increasingly available. In addition, Table 7.1 demonstrates that the prevalences of GI nematodes, *F. hepatica*, *D. viviparus* and *N. caninum* in adult cattle in Western Europe are high. Many herds will thus be infected with multiple parasitic species. Consequently, it would be of great interest to measure the effect of concurrent infections with multiple parasitic and/or non-parasitic species. Previously, an attempt has been made to assess the impact of concurrent infections with GI nematodes and *F. hepatica*. When considered separately, an increase in the antibody levels against *O. ostertagi* and *F. hepatica* over the interquartile range was associated with a decrease in milk yield of 0.9 and 0.7 kg/cow per day, respectively (chapter 4; Charlier *et al.*, 2007b). However, a simultaneous increase over the interquartile range of the antibody levels against both parasites was associated with a decrease of 1.3 kg/cow per day, which is less than the sum of the 2

separate associations. VanLeeuwen *et al.* (2006) used a similar approach by measuring the associations of seropositivity status of 4 different pathogens (BVD virus, BL virus, MAP and *N. caninum*) with milk-yield, but did not consider helminth infections. The results of the present thesis indicate that helminth infections should be incorporated in such studies.

Table 7.1. Reported prevalences (prev.) of infections with *O. ostertagi*, *D. viviparus*, *F. hepatica* and *N. caninum* in adult cattle in Western Europe.

Species	Country	Prev.	Method	Reference
O. ostertagi	Belgium	91 %	Individual worm counts	(Agneessens *et al.*, 2000)
	Ireland	57 %	Individual worm counts	(Murphy *et al.*, 2006)
	Netherlands	96 %	Individual worm counts	(Borgsteede *et al.*, 2000)
D. viviparus	Ireland	14 %	Individual coprology	(Murphy *et al.*, 2006)
	Netherlands	72 %	Herd level coprology	(Eysker *et al.*, 1994)
F. hepatica	Belgium	57 %	Herd level serology	(Lonneux *et al.*, 1999-2000)
	England	48 %	Herd level serology	(Salimi-Bejestani *et al.*, 2005)
	France	13 %	Individual coprology	(Mage *et al.*, 2002)
	Ireland	65 %	Individual liver necropsy	(Murphy *et al.*, 2006)
	Portugal	48 %	Herd level serology	(Conceição *et al.*, 2004)
	Switzerland	18 %	Individual prevalence by Bayesian approach	(Rapsch *et al.*, 2006)
	Wales	86 %	Herd level serology	(Salimi-Bejestani *et al.*, 2005)
N. caninum	Germany	49 %	Herd level serology	(Bartels *et al.*, 2006a)
	Netherlands	76 %	Herd level serology	(Bartels *et al.*, 2006a)
	Spain	63 %	Herd level serology	(Bartels *et al.*, 2006a)
	Sweden	16 %	Herd level serology	(Bartels *et al.*, 2006a)

There exist indications that infections with GI nematodes are related with the occurrence of metabolic disease. Sithole (2005) found that the odds of developing subclinical ketosis after calving was positively associated with the *Ostertagia* ODR determined at the end of the previous lactation. In addition, it has been suggested that *Ostertagia* infection increases the general susceptibility to disease (Hawkins, 1993). The use of the *O. ostertagi* ELISA by investigating the associations between the *Ostertagia* ODR and disease occurrence or immunity development could lead to new insights in these subjects. A possible approach would be to examine the relationship between *Ostertagia* ODR and immune response to a heterologous antigen. Such knowledge would be of value to develop GI-nematode control strategies to enhance the herd's response to vaccination programmes.

5. The use of milk ELISAs and geographical information systems to determine the regional economic importance of parasitic diseases

Climatic factors such as rainfall and temperature have a large impact on the epidemiology of many parasites. GIS and remote sensing (RS) are emerging tools in epidemiological research to assess the impact of environmental and climatic features on parasite distribution. GIS is commonly defined as "an extensive compilation of resources to collect, save, reclaim and visualize spatial data" (Burrough, 1986). Besides ground-measured variables and analogue maps, one possible source of spatial information is RS. By satellite measurement, information can be obtained at various resolutions on temperature, rainfall, vegetation and environmental features. GIS and RS can be used in decision support by studying environmental features that influence parasite distribution, predicting parasite occurrence and monitoring animal diseases (Cringoli *et al.*, 2005). The developed methodologies are now considered robust enough to be included routinely in spatial epidemiology studies and decision support. However, until now they have not much been used to study enzootic diseases in temperate-climate regions (Hendrickx *et al.,* 2004).

Despite the knowledge that parasite distribution is affected by climatic and environmental factors, the relationship with these factors has insufficiently been investigated to produce spatial distribution models or forecasting systems. *F. hepatica* is until now the only parasite of dairy cattle for which such systems have successfully been applied in some temperate-climate regions (e.g. Ross, 1975). Although the effect of climate and environment on the distribution of other parasites may be weaker, the use of GIS and parasitological ELISAs on bulk-tank milk would be highly suitable to study possible spatial and temporal patterns. Bulk-tank milk samples can be easily (and repeatedly) collected from a large number of randomly selected herds, allowing the production of high-precision and representative maps of the parasite distribution in a given region. We have shown that significant associations occur between *O. ostertagi* ELISA results and productivity. If such relationships could also be determined for

other parasitological ELISAs, it would not only be possible to map the distribution of infection, but also its economic importance.

6. Conclusion

The present thesis has contributed to the *Ostertagia*-specific antibody levels in bulk-tank milk in relation to management and production parameters. An *Ostertagia* ELISA on bulk-tank milk can be highly relevant to monitor GI-nematode infections in the adult dairy herd and to prevent GI-nematode associated production losses. However, the *Ostertagia*-specific antibody level in bulk-tank milk can also be influenced by non-parasitic factors (e.g. mastitis, changes in the milking-herd composition and the relative milk yield of cows contributing the bulk-tank). In addition, production traits are affected by many unknown factors. Therefore, thresholds that predict production gains after treatment will only be indicative. For research purposes, parasitological milk ELISAs offer convenient tools to study the "effect" of subclinical parasitic infections on productivity; the inter-relationships between parasitic infections and other diseases or immune responses and the effect of spatial and climatic factors on the distribution and economic importance of parasitic infections.

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Summary

Summary

Infection of cattle with gastrointestinal (GI) nematodes is a major constraint on production worldwide. Traditionally, infections with GI nematodes were considered to be mainly important in first-season grazing calves. GI-nematode infections in older cattle were for a long time considered to be of limited importance because of the absence of clinical symptoms and the lower fecal egg counts usually found in these animals. However, the prevalence of GI nematodes in adult cows is still very high, with *Ostertagia ostertagi* as the most important species. Moreover, it has been demonstrated that subclinical GI-nematode infections can cause a reduced productivity of adult dairy cows. This, together with the availability of highly efficacious anthelmintics with a zero withdrawal time for milk has generated a new interest the importance of GI-nematode infections in adult dairy cows.

The literature review (**chapter 1**) addresses the diagnosis, impact on production and control of GI-nematode infections in adult dairy cows. Several diagnostic parameters (fecal egg counts, serum pepsinogen and specific-antibody levels) have been evaluated in their ability to estimate the infection level in adult cows. The only parameter showing potential is the *Ostertagia*-specific antibody level and especially determination of these antibodies in milk is considered to be cost-effective. The most reported effect on production of GI-nematode infection in dairy cows is a reduced milk yield. The mean milk-yield response after anthelmintic treatment is 0.4 to 0.6 kg/cow per day, but large variations in treatment responses are observed between different trials. The level of GI-nematode infection is considered to be a determining factor in the magnitude of the treatment response. Pour-on formulations of eprinomectin and moxidectin are the most used anthelmintic drugs in dairy cows because they have the highest efficacy and a zero withdrawal time for milk. Due to the increasing concerns on the development of anthelmintic resistance, it is currently proposed to base GI-nematode control on selective treatments of only the animals/herds where infection-associated production losses occur and on non-chemotherapeutic options such as control through grazing

management. However, a problem is how to identify the animals or herds where GI nematodes are causing a production loss.

Therefore, it was considered relevant to investigate whether *Ostertagia*-specific antibody levels in bulk-tank milk can be used to monitor GI-nematode infections in a dairy herd and to identify the herds where a production gain after anthelmintic treatment is expected. The overall objective of this thesis was to study the epidemiology and impact on production of GI-nematode infections in dairy cows by the use of anti-*O. ostertagi* antibodies in bulk-tank milk.

In **chapter 2** border effects and the repeatability of an indirect *O. ostertagi* milk ELISA and the effects of different factors such as storage, skimming and freeze-thaw cycles of the milk samples were investigated. The ELISA results were expressed as optical-density ratios (ODR). The border-effects trial showed that significant border effects can occur. The repeatability trial was conducted over 3 days. A graphical technique to assess the repeatability over a large number of ELISA plates measured over different days was developed. From these graphs, it was obvious that the ODR values obtained on the third day were deviating from the values on the first and second day. On the third day, also abnormal control values were observed. When the control values were normal, 94 % of the variability was explained by the milk sample and 6 % by assay variability. The expected 95 % range of the difference of 2 ODR readings of the same sample on the same plate and the same sample on different plates was –0.14 to 0.14 and -0.16 to 0.16. No extra variability was observed when samples were tested on a different day, however these results are only based on the measurement of 2 days. Storage for 2-4 days at 4 °C, using whole milk instead of skimmed milk and up to 2 extra freeze-thaw cycles of the milk samples did not significantly affect the test results.

In **chapter 3**, the effect of an experimentally induced mastitis on the ODR results of an *O. ostertagi* milk ELISA was investigated. Twenty-five cows that were naturally infected with GI nematodes, were inoculated in their left udder quarters with *Escherichia coli* P4:O32 and quarter milk samples were collected at several intervals from 24 h before until 144 h after experimental infection. The effect of the contribution of milk from 1 or more infected quarters on the bulk-tank milk ODR was estimated, based

on a titration experiment. The mean *O. ostertagi* antibody level in the milk samples of the infected udder quarters was significantly higher than of the uninfected udder quarters at each sampling time post-infection. The largest difference was observed at 24 h post-infection with a mean difference of 0.251 ODR. After the infection, there was also a significant increase in total IgG levels with the largest difference being observed at 24 h post-infection. Highly significant correlation coefficients were observed between the *Ostertagia* ODR, total IgG ODR, Na^+ and Cl^- ion concentration and log transformed somatic-cell counts at 24 h post-infection. The results demonstrate that an acute mastitis causes a flow of specific and non-specific antibodies from serum to milk with a subsequent increase in the *O. ostertagi* ODR values. The effect of the contribution of milk from infected quarters on the bulk-tank milk *Ostertagia* ODR was estimated to be minor if the relative number of infected quarters is small (< 3 %).

In **chapter 4,** the associations were investigated between anti-*O. ostertagi* antibody levels in bulk-tank milk and milk-production parameters. Bulk-tank milk samples of 2,553 Flemish dairy herds were obtained in spring and 2,104 of these herds were sampled a second time in autumn. The association of the bulk-tank milk *Ostertagia* ODR on 3 different production parameters (kg milk, % and kg milk-fat, % and kg milk-protein) was assessed by multivariable linear-regression models on the herds for which production data were available (*n*= 1,063 and 867 in spring and autumn, respectively). The mean and standard deviation for ODR_{autumn} (0.972 ± 0.238) were higher than for ODR_{spring} (0.825 ± 0.201). An increase in ODR_{spring} and ODR_{autumn} from the 25^{th} to the 75^{th} percentile was associated with a drop in the annual milk yield of 1.1 and 0.9 kg/cow per day, respectively. When a herd's bulk-tank milk ODR increased between spring and autumn with 0.142, it produced on average 0.4 kg/cow per day less in September than in April, in comparison with herds where the ODR did not increase. A significant negative association was found between ODR_{autumn} and milk-protein % averaged over the period of a year, but no association was found with milk-fat % averaged over a year. When the milk-protein and milk-fat production of September were expressed in kg, an increase in ODR_{autumn} from the 25^{th} to the 75^{th}

percentile was associated with a decrease of 0.037 and 0.042 kg/cow per day, respectively.

In **chapter 5**, a cross-sectional questionnaire survey was performed to study the associations between herd-management factors and the *Ostertagia*-specific antibody levels in bulk-tank milk. At the end of the grazing season, information concerning general herd factors, pasture management and anthelmintic treatment strategy was obtained from 956 herds. A bulk-tank milk sample was obtained from 779 out of the 956 herds and the *Ostertagia* ODR was determined. The associations between *Ostertagia* ODR and herd-management factors were studied by 2 linear-regression models. The first model evaluated the "effect" of general herd factors and the level of the cows' exposure to pasture. Large-sized herds (> 60 cows) had significantly lower ODRs as compared to medium- or small-sized herds (< 30 cows). Herds with only dairy cows had lower ODRs than herds with both dairy and beef cows. An increased exposure to pasture of the cows was associated with higher ODRs. The second model was built to evaluate the "effect" of pasture-management factors and anthelmintic-treatment strategy. Later turnout on pasture and mowing were both significantly associated with lower ODRs. Herds where the cows' daily grazing time was restricted tended to have lower ODRs than cows that grazed 24 h per day. An increased exposure to pasture of the heifers was significantly associated with higher ODRs. No associations were found between ODR and calf-related management factors, anthelmintic-treatment strategy, time of turn-in, rotational grazing type or stocking rate. Postponing turnout on pasture, mowing and restricting the grazing time per day are non-chemotherapeutic control measures that can be applied to reduce the infection level with GI nematodes on dairy farms.

The objectives of **chapter 6** were (1) to determine the effect of a herd treatment of pastured dairy herds with eprinomectin in autumn on the anti-*O. ostertagi* bulk-tank milk antibody level; (2) to determine the overall effect of this treatment on 3 milk-production parameters (kg milk, milk-protein % and milk-fat %) and (3) to investigate the value of the pre-treatment *Ostertagia*-specific bulk-tank milk antibody level to predict the milk-yield response after anthelmintic treatment. One-hundred and nineteen herds in Flanders (Belgium) were randomly assigned to a

treatment with eprinomectin or a placebo in October. Bulk-tank milk samples were collected monthly from 2 months before until 6 months after the treatment and the *Ostertagia* ODR was determined with an ELISA. The treatment effect over the 4 months following treatment on the production parameters was estimated by mixed models with herd as a random effect. The treatment effect on milk yield was also investigated within 6 categories of the pre-treatment ODR. The ODR values were lower in the eprinomectin group than in the placebo group at each time point after treatment. The overall effect on milk yield was estimated at 1.2 kg/cow per day, whereas no effect on the milk-protein % and milk-fat % was observed. Herds in the highest pre-treatment ODR category (> 0.84) had a positive milk-yield response of 4.0 kg/cow per day (95 % confidence interval: 1.0; 7.0), while the 95 % confidence intervals of the milk-yield response in the other categories all included zero. This study demonstrates that treatment with eprinomectin of pastured dairy cows in autumn will lower the *Ostertagia*-specific bulk-tank milk antibody level during the stabling period and can result in a consistent increase in milk yield. An indirect *O. ostertagi* ELISA on bulk-tank milk can to some extent identify the herds where a positive milk-yield response after an anthelmintic treatment is expected.

In **chapter 7**, the possible uses of the *Ostertagia* ELISA by veterinarians and researchers are discussed. It is proposed to use the *Ostertagia*-specific antibody level in bulk-tank milk at the end of the grazing season to evaluate the herd's exposure to GI nematodes during the finished grazing period. Moreover, this parameter can be incorporated in the decision-making process whether anthelmintic treatment around stabling is likely to result in a production gain. The definition of a clear ODR threshold "to treat or not to treat" is less evident because production traits and *Ostertagia* ODRs are influenced by many unknown and unverifiable factors. In the future, *Ostertagia*-specific antibody levels in individual milk samples could be a suitable parameter to detect the animals within a herd that suffer from GI-nematode associated production losses and enable selective treatment programmes within a herd. The latter aspect, together with studies on the effects of concurrent subclinical infections with different (non-)parasitic species on productivity, on the

associations between the *Ostertagia* ODR and other infectious/metabolic diseases and on the use of parasitological milk ELISAs in geographical information systems offer a wide range of future research applications.

Samenvatting

Samenvatting

Infecties met gastrolintestinale nematoden (GIN) bij runderen veroorzaken wereldwijd belangrijke productieverliezen. In het verleden werden GIN-infecties vooral als een probleem beschouwd bij eerste weideseizoenskalveren. Bij volwassen koeien hechtte men er weinig belang aan, wegens het ontbreken van klinische symptomen en de doorgaans lage fecale eitellingen bij deze leeftijdsgroep. Nochtans is gebleken dat de prevalentie van GIN bij volwassen melkkoeien zeer hoog is, met *Ostertagia ostertagi* als het belangrijkste species. Bovendien werd aangetoond dat subklinische GIN-infecties een negatieve invloed kunnen hebben op de melkgift. Samen met de beschikbaarheid van uiterst effectieve anthelminthica met een nul-dagen wachttijd voor melk, heeft dit de interesse aangewakkerd voor het belang van GIN bij volwassen melkkoeien.

In **hoofdstuk 1** wordt een overzicht gegeven van de bestaande literatuur over de diagnose, impact op productie en controle van GIN-infecties bij melkkoeien. Uit verschillende diagnostische parameters (fecale eitelling, serum pepsinogeen en specifieke antistofgehaltes) worden bij volwassen koeien enkel *Ostertagia*-specifieke antistofgehaltes als een beloftevolle parameter beschouwd voor het inschatten van het infectieniveau met GIN. Vooral de bepaling van deze antistoffen in melk wordt als rendabel beschouwd. Het meest vermelde effect van GIN-infecties op de productiviteit van melkkoeien is een verminderde melkgift. Het gemiddelde effect van een anthelminthische behandeling op de melkgift is 0.4 à 0.6 kg/koe per dag, maar er zijn grote verschillen in de behandelingseffecten tussen verschillende studies. Het GIN-infectieniveau wordt beschouwd als een bepalende factor in de grootte van het behandelingseffect. Pour-on formulaties van eprinomectine en moxidectine zijn de meest gebruikte anthelminthica bij melkkoeien omdat ze uiterst effectief zijn en een nul-dagen wachttijd voor melk hebben. Door de toenemende bezorgdheid over de ontwikkeling van anthelminthicum-resistentie bij GIN, worden momenteel controlemaatregelen voorgesteld die gebaseerd zijn op een selectieve behandeling van enkel de dieren/bedrijven met productieverliezen ten

gevolge van GIN-infecties en op niet-chemotherapeutische opties zoals controle door weidemanagement. Tot op heden bestaat er echter geen parameter om de dieren of bedrijven met parasiet-geïnduceerde productieverliezen te identificeren.

Daarom werd besloten om te onderzoeken of het *Ostertagia*-specifieke antistofgehalte in tankmelk gebruikt kan worden om het belang van GIN-infecties op melkveebedrijven te evalueren en om de bedrijven te identificeren waar een productieverhoging na anthelminthische controle verwacht wordt. Het algemene objectief van deze thesis was de studie van de epidemiologie en de impact op productie van GIN-infecties bij melkkoeien a.h.v. anti-*O. ostertagi* antistoffen in tankmelk.

In **hoofdstuk 2** werden randeffecten en de herhaalbaarheid van een indirecte *O. ostertagi* melk ELISA onderzocht. Bovendien werden de effecten op de ELISA resultaten nagegaan van verschillende voorbehandelingen van de melkstalen zoals het bewaren bij 4 °C, afromen en vries-dooi cycli. De ELISA resultaten werden uitgedrukt als een optische densiteit ratio (ODR). Er werd aangetoond dat significante randeffecten aanwezig kunnen zijn. De herhaalbaarheid werd bestudeerd gedurende 3 dagen. Er werd een grafische techniek ontwikkeld om de herhaalbaarheid te bepalen over een groot aantal ELISA platen die getest werden gedurende verschillende dagen. Uit deze grafieken bleek dat de ODRs van de derde dag, afweken van de ODRs op de eerste en tweede dag. Op de derde dag werden ook abnormale waarden van de controlestalen vastgesteld. Toen de waarden van de controlestalen niet afweken, werd 94 % van de variabiliteit verklaard door het melkstaal en 6 % door test variabiliteit. Het verwachte 95 % interval van het verschil van 2 ODR metingen van hetzelfde staal op dezelfde plaat en hetzelfde staal op verschillende platen was -0.14 tot 0.14 en -0.16 tot 0.16. Er werd geen bijkomende variabiliteit vastgesteld wanneer de stalen op een andere dag getest werden, maar dit resultaat is gebaseerd op metingen gedurende slechts 2 dagen. Het bewaren van de melkstalen gedurende 2-4 dagen bij 4 °C, volledige melk gebruiken i.p.v. afgeroomde melk en tot 2 extra vries-dooi cycli hadden geen significant effect op de ODRs.

In **hoofdstuk 3** werd het effect nagegaan van een experimenteel geïnduceerde mastitis op de test resultaten van een *O. ostertagi* melk

ELISA. Vijfentwintig koeien, natuurlijk geïnfecteerd met GIN, werden in de linker uierkwartieren geïnoculeerd met *Escherichia coli* P4:O32. Er werden kwartiermelkstalen verzameld op verschillende tijdstippen van 24 u voor tot 144 u na de experimentele infectie. Het effect van de bijdrage van 1 geïnfecteerd kwartier op de tankmelk ODR werd geschat a.h.v. een titratie-experiment. Het gemiddelde *Ostertagia*-specifieke antistofgehalte van de melkstalen uit de geïnfecteerde uierkwartieren was significant hoger dan uit de niet-geïnfecteerde kwartieren op elk tijdstip na de infectie. Het grootste verschil werd vastgesteld 24 u na infectie met een gemiddelde verschil van 0.251 ODR. Na de infectie was er ook een significante toename in de totale IgG gehaltes in de melk met maximale waarden na 24 u. Significante correlaties werden vastgesteld 24 u na infectie tussen het *O. ostertagi*-specifieke antistofgehalte, totale IgG gehalte, Na^+ en Cl^- ion concentratie en het log getransformeerde somatisch celgetal. Deze resultaten demonstreren dat een acute mastitis een stroom van specifieke en niet-specifieke antistoffen van serum naar melk veroorzaken met een toename van de *O. ostertagi* ODRs tot gevolg. Het effect van melk van geïnfecteerde kwartieren op de tankmelk ODR werd als klein geschat, op voorwaarde dat het proportioneel aantal geïnfecteerde kwartieren klein is (< 3 %).

In **hoofdstuk 4** werden de verbanden onderzocht tussen het *Ostertagia*-specifieke antistofgehalte in tankmelk en melkproductieparameters. Tankmelkstalen van 2553 Vlaamse melkveebedrijven werden verzameld in de lente en 2104 van deze bedrijven werden een tweede maal bemonsterd in de herfst. Van deze bedrijven waren er productiedata beschikbaar voor *n*= 1063 en 867 in respectievelijk de lente en de herfst. Het verband tussen de *Ostertagia* ODR in tankmelk en 3 verschillende productieparameters (kg melk, % and kg melkvet, % and kg melkeiwit) werd bepaald via multivariabele lineaire regressie. Het gemiddelde en de standaardafwijking van ODR_{herfst} (0.972 ± 0.238) waren hoger dan van ODR_{lente} (0.825 ± 0.201). Een toename in de ODR_{lente} en ODR_{herfst} van het 25^{ste} tot het 75^{ste} percentiel was geassocieerd met een daling in de jaarproductie van melk met respectievelijk 1.1 en 0.9 kg/koe per dag. Wanneer op een bedrijf het *Ostertagia*-specifieke antistofgehalte toenam tussen de lente en de herfst met 0.142 ODR, produceerde dit bedrijf

gemiddeld 0.4 kg/koe per dag minder in september dan in april in vergelijking met bedrijven waar het *Ostertagia*-specifieke antistofgehalte niet toenam. Er werd tevens een significant negatief verband aangetoond tussen ODR_{herfst} en het jaargemiddelde melkeiwit %, maar niet met het jaargemiddelde melkvet %. Wanneer de eiwit- en vetproductie in september uitgedrukt werden in kg, was een stijging van ODR_{herfst} van het 25^{ste} tot het 75^{ste} percentiel geassocieerd met een daling van respectievelijk 0.037 en 0.042 kg/koe per dag.

In **hoofdstuk 5** werd een cross-sectionele studie uitgevoerd om de verbanden te onderzoeken tussen het *Ostertagia*-specifieke antistofgehalte in tankmelk en bedrijfsmanagementfactoren. Op het einde van het weideseizoen werd via een enquête op 956 bedrijven informatie verzameld over algemene bedrijfskenmerken, weidebeheer en anthelminthische behandelingsstrategie. Van de 956 bedrijven kon er voor 779 een tankmelkstaal onderzocht worden met een *O. ostertagi* ELISA. De verbanden tussen *Ostertagia* ODR en managementfactoren werden onderzocht a.h.v. 2 lineaire regressiemodellen. In het eerste model werd het verband nagegaan met algemene bedrijfskenmerken en de mate van weidegang van de koeien. Grote bedrijven (> 60 koeien) en bedrijven met enkel melkvee hadden een significant lagere ODR dan respectievelijk middelgrote of kleine bedrijven (< 30 koeien) en gemengde bedrijven (melk- en vleesvee). Bedrijven met weidegang van de koeien hadden een significant hogere ODR dan bedrijven met zero-grazing of een kleine uitloop. In het tweede model werd het verband geëvalueerd met weidebeheer en anthelminthische behandelingsstrategie. Het uitstellen van het uitweiden en maaien van de weide waren significant geassocieerd met een lagere ODR. Tevens was er een tendens van een lagere ODR bij het beperken van de dagelijkse graastijd van de koeien. Een verhoogde weidegang van de vaarzen was significant geassocieerd met een hogere ODR. Er werd geen verband gevonden tussen ODR en kalf-gerelateerde managementfactoren, anthelminthische behandelingsstrategie, tijdstip van opstallen, rotationeel grazen of weidebezettingsdensiteit. Het uitstellen van het uitweiden, maaien van de weide en het beperken van de dagelijkse graastijd kunnen dus gebruikt worden als niet-

chemotherapeutische controlemaatregelen om het infectieniveau met GIN te verminderen.

De objectieven van **hoofdstuk 6** waren (1) het bepalen van het effect van een groepsbehandeling in de herfst met eprinomectine op het anti-*O. ostertagi* antistofgehalte in de tankmelk; (2) het bepalen van het globale effect van deze behandeling op 3 melkproductieparameters (kg melk, melkeiwit %, melkvet %) en (3) het onderzoeken van de waarde van het *Ostertagia*-specifieke antistofgehalte in de tankmelk vóór behandeling om de melkproductierespons na behandeling te voorspellen. Honderdnegentien bedrijven in Vlaanderen werden willekeurig toegewezen aan een groepsbehandeling met eprinomectine of een placebo in oktober. Vanaf 2 maanden voor t.e.m. 6 maanden na de behandeling werden maandelijks tankmelkstalen verzameld en onderzocht met een *O. ostertagi* ELISA. Het behandelingseffect gedurende de 4 maanden na behandeling op de productieparameters werd geschat a.h.v. gemengde modellen met bedrijf als random effect. Vervolgens werd het behandelingseffect nagegaan binnen 6 categorieën op basis van de *Ostertagia* ODR voor behandeling. De ODRs waren lager in de eprinomectine-groep dan in de placebo-groep op elk gemeten tijdstip na behandeling. Het globale effect van de behandeling op de melkgift werd geschat op 1.2 kg/koe per dag. Er werd geen effect vastgesteld op het melkeiwit % of melkvet %. Bedrijven in de hoogste ODR-categorie (> 0.84) reageerden op de behandeling met een verhoging van de melkgift met 4.0 kg/koe per dag (95 % betrouwbaarheidsinterval: 1.0; 7.0), terwijl de 95 % betrouwbaarbaarheidsintervallen van de andere categorieën nul bevatten. De conclusies van deze studie waren dat een groepsbehandeling in de herfst met eprinomectine een verlaging van de *Ostertagia* ODR in tankmelk gedurende de opstalperiode tot gevolg heeft en kan resulteren in een toegenomen melkgift. Een *O. ostertagi* ELISA op tankmelk kan in zekere mate de bedrijven identificeren waar een positief effect op de melkgift na anthelminthische behandeling verwacht wordt.

Tenslotte worden in de algemene discussie (**hoofdstuk 7**) de mogelijke toepassingen van de *Ostertagia* ELISA door dierenartsen en onderzoekers besproken. Er wordt voorgesteld om het *Ostertagia*-specifieke antistofgehalte in de tankmelk op het einde van het weideseizoen te

gebruiken om de blootstelling aan GIN gedurende het voorbije weideseizoen te bepalen. Bovendien kan deze parameter gebruikt worden als hulpmiddel om te bepalen of een anthelminthische behandeling bij het opstallen zal resulteren in een verhoogde melkgift. Het bepalen van een duidelijke ODR-drempelwaarde "behandelen of niet behandelen" was echter niet mogelijk omdat zowel de productieparameters als de *Ostertagia* ODR beïnvloed worden door onbekende en oncontroleerbare factoren. In de toekomst zou het *Ostertagia*-specifieke antistofgehalte in individuele melkstalen misschien gebruikt kunnen worden om binnen een bedrijf de dieren te identificeren met productieverliezen t.g.v. GIN-infecties. Dit aspect, samen met studies naar het effect van gelijktijdige subklinische infecties met verschillende (niet-)parasitaire species op productiviteit, naar de verbanden tussen *Ostertagia* ODR en andere infectieuze of metabole ziekten en naar regionale verschillen in het economisch belang van GIN-infecties bieden ruime mogelijkheden voor toekomstige onderzoekstoepassingen.